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(54) Title: A METHOD FOR IDENTIFICATION, ISOLATION AND PRODUCTION OF ANTIGENS TO A SPECIFIC PATHOGEN

(57) Abstract: Described is a method for identification, isolation and production of hyperimmune serum-reactive antigens from a specific pathogen, a tumor, an allergen or a tissue or host prone to autoimmunity, said antigens being suited for use in a vaccine for a given type of animal or for humans, which is characterized by the following steps: - providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - providing at least one expression library of said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - screening said at least one expression library with said antibody preparation, - identifying antigens which bind in said screening to antibodies in said antibody preparation, - screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity, - identifying the hyperimmune serum-reactive antigen portion of said identified antigens and which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera and - optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods.

WO 02/059148 A2

A method for identification, isolation and production of antigens to a specific pathogen

The invention relates to a method for identification, isolation and production of antigens to a specific pathogen as well as new antigens suitable for use in a vaccine for a given type of animal or for humans.

Vaccines can save more lives (and resources) than any other medical intervention. Owing to world-wide vaccination programmes the incidence of many fatal diseases has been decreased drastically. Although this notion is valid for a whole panel of diseases, e.g. diphtheria, pertussis, measles and tetanus, there are no effective vaccines for numerous infectious disease including most viral infections, such as HIV, HCV, CMV and many others. There are also no effective vaccines for other diseases, infectious or non-infectious, claiming the lifes of millions of patients per year including malaria or cancer. In addition, the rapid emergence of antibiotic-resistant bacteria and microorganisms calls for alternative treatments with vaccines being a logical choice. Finally, the great need for vaccines is also illustrated by the fact that infectious diseases, rather than cardiovascular disorders or cancer or injuries remain the largest cause of death and disability in the world.

Several established vaccines consist of live attenuated organisms where the risk of reversion to the virulent wild-type strain exists. In particular in immunocompromised hosts this can be a live threatening scenario. Alternatively, vaccines are administered as a combination of pathogen-derived antigens together with compounds that induce or enhance immune responses against these antigens (these compounds are commonly termed adjuvant), since these subunit vaccines on their own are generally not effective.

Whilst there is no doubt that the above vaccines are valuable medical treatments, there is the disadvantage that, due to their complexity, severe side effects can be evoked, e.g. to antigens that are contained in the vaccine that display cross-reactivity with molecules expressed by cells of vaccinated individuals. In addition, existing requirements from regulatory authorities, e.g.

the World Health Organization (WHO), the Food and Drug Administration (FDA), and their European counterparts, for exact specification of vaccine composition and mechanisms of induction of immunity, are difficult to meet.

Some widely used vaccines are whole cell-vaccines (attenuated bacteria or viruses (e.g. Bacille Calmette-Guerin (BCG) (tuberculosis), Measles, Mumps, Rubella, Oral Polio Vaccine (Sabin), killed bacteria or viruses (e.g. Pertussis, Inactivated polio vaccine (Salk)), subunit-vaccines (e.g. Toxoid (Diphtheria, Tetanus)), Capsular polysaccharide (H. influenzae type B), Yeast recombinant subunit (Hepatitis B surface protein).

A vaccine can contain a whole variety of different antigens. Examples of antigens are whole-killed organisms such as inactivated viruses or bacteria, fungi, protozoa or even cancer cells. Antigens may also consist of subfractions of these organisms/tissues, of proteins, or, in their most simple form, of peptides. Antigens can also be recognized by the immune system in form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used since for example cytotoxic T-cells (CTL) recognize antigens in form of short usually 8-11 amino acids long peptides in conjunction with major histocompatibility complex (MHC). B-cells can recognize linear epitopes as short as 4-5 amino acids, as well as three dimensional structures (conformational epitopes). In order to obtain sustained, antigen-specific immune responses, adjuvants need to trigger immune cascades that involve all cells of the immune system necessary. Primarily, adjuvants are acting, but are not restricted in their mode of action, on so-called antigen presenting cells (APCs). These cells usually first encounter the antigen(s) followed by presentation of processed or unmodified antigen to immune effector cells. Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response. The adjuvant may also locally retain antigens and co-injected other factors. In addition the adjuvant may act as a chemoattractant for other immune cells or may act locally and/or systemically as a stimulating agent for the immune system.

Antigen presenting cells belong to the innate immune system, which has evolved as a first line host defence that limits infection early after exposure to microorganisms. Cells of the innate immune system recognize patterns or relatively non-specific structures expressed on their targets rather than more sophisticated, specific structures which are recognized by the adaptive immune system. Examples of cells of the innate immune system are macrophages and dendritic cells but also granulocytes (e.g. neutrophiles), natural killer cells and others. By contrast, cells of the adaptive immune system recognize specific, antigenic structures, including peptides, in the case of T-cells and peptides as well as three-dimensional structures in the case of B-cells. The adaptive immune system is much more specific and sophisticated than the innate immune system and improves upon repeated exposure to a given pathogen/antigen. Phylogenetically, the innate immune system is much older and can be found already in very primitive organisms. Nevertheless, the innate immune system is critical during the initial phase of antigenic exposure since, in addition to containing pathogens, cells of the innate immune system, i.e. APCs, prime cells of the adaptive immune system and thus trigger specific immune responses leading to clearance of the intruders. In sum, cells of the innate immune system and in particular APCs play a critical role during the induction phase of immune responses by a) containing infections by means of a primitive pattern recognition system and b) priming cells of the adaptive immune system leading to specific immune responses and memory resulting in clearance of intruding pathogens or of other targets. These mechanisms may also be important to clear or contain tumor cells.

The antigens used for such vaccines have often been selected by chance or by easiness of availability. There is a demand to identify efficient antigens for a given pathogen or - preferably - an almost complete set of all antigens of a given pathogen which are practically (clinically) relevant. Such antigens may be preferred antigen candidates in a vaccine.

It is therefore an object of the present invention to comply with these demands and to provide a method with which such antigens may be provided and with which a practically complete set of an-

tigens of e.g. a given pathogen may be identified with a given serum as antibody source. Such a method should also be suitable for rapidly changing pathogens which evolve a fast resistance against common drugs or vaccines. The method should also be applicable to identify and isolate tumor antigens, allergens, auto-immune antigens.

Therefore, the present invention provides a method for identification, isolation and production of hyperimmune serum-reactive antigens from a specific pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity, especially from a specific pathogen, said antigens being suited for use in a vaccine for a given type of animal or for humans, said method being characterized by the following steps:

- providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity,
- providing at least one expression library of said specific pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity,
- screening said at least one expression library with said antibody preparation,
- identifying antigens which bind in said screening to antibodies in said antibody preparation,
- screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity,
- identifying the hyperimmune serum-reactive antigen portion of said identified antigens which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera and
- optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods.

This method is also suitable in general for identifying a practically complete set of hyperimmune serum-reactive antigens of a specific pathogen with given sera as antibody sources, if at

least three different expression libraries are screened in a pathogen/antigen identification programme using the method according to the present invention. The present invention therefore also relates to a method for identification, isolation and production of a practically complete set of hyperimmune serum-reactive antigens of a specific pathogen, said antigens being suited for use in a vaccine for a given type of animal or for humans, which is characterized by the following steps:

- providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen,
- providing at least three different expression libraries of said specific pathogen,
- screening said at least three different expression libraries with said antibody preparation,
- identifying antigens which bind in at least one of said at least three screenings to antibodies in said antibody preparation,
- screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen,
- identifying the hyperimmune serum-reactive antigen portion of said identified antigens which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera,
- repeating said screening and identification steps at least once,
- comparing the identified hyperimmune serum-reactive antigens identified in the repeated screening and identification steps with the identified hyperimmune serum-reactive antigens identified in the initial screening and identification steps,
- further repeating said screening and identification steps, if at least 5% of the hyperimmune serum-reactive antigens have been identified in the repeated screening and identification steps only, until less than 5 % of the hyperimmune serum-reactive antigens are identified in a further repeating step only to obtain a complete set of hyperimmune serum-reactive antigens of a specific pathogen and
- optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by

chemical or recombinant methods.

The method according to the present invention mainly consists of three essential parts, namely 1. identifying hyperimmune serum sources containing specific antibodies against a given pathogen, 2. screening of suitable expression libraries with a suitable antibody preparation wherein candidate antigens (or antigenic fragments of such antigens) are selected, and - 3. in a second screening round, wherein the hyperimmune serum-reactive antigens are identified by their ability to bind to a relevant portion of individual antibody preparations from individual sera in order to show that these antigens are practically relevant and not only hyperimmune serum-reactive, but also widely immunogenic (i.e. that a lot of individual sera react with a given antigen). With the present method it is possible to provide a set of antigens of a given pathogen which is practically complete with respect to the chosen pathogen and the chosen serum. Therefore, a bias with respect to "wrong" antigen candidates or an incomplete set of antigens of a given pathogen is excluded by the present method.

Completeness of the antigen set of a given pathogen within the meaning of the present invention is, of course, dependent on the completeness of the expression libraries used in the present method and on the quality and size of serum collections (number of individual plasmas/sera) tested , both with respect to representability of the library and usefulness of the expression system. Therefore, preferred embodiments of the present method are characterized in that at least one of said expression libraries is selected from a ribosomal display library, a bacterial surface library and a proteome.

A serum collection used in the present invention should be tested against a panel of known antigenic compounds of a given pathogen, such as polysaccharide, lipid and proteinaceous components of the cell wall, cell membranes and cytoplasma, as well as secreted products. Preferably, three distinct serum collections are used: 1. With very stable antibody repertoire: normal adults, clinically healthy people, who overcome previous encounters or currently carriers of e.g. a given pathogen without acute disease and symptoms, 2. With antibodies induced acutally by the presence

of the pathogenic organism: patients with acute disease with different manifestations (e.g. S. aureus sepsis or wound infection, etc.), 3. With no specific antibodies at all (as negative controls): 5-8 months old babies who lost the maternally transmitted immunoglobulins 5-6 months after birth. Sera have to react with multiple pathogen-specific antigens in order to consider hyperimmune for a given pathogen (bacteria, fungus, worm or otherwise), and for that relevant in the screening method according to the present invention.

In the antigen identification programme for identifying a complete set of antigens according to the present invention, it is preferred that said at least three different expression libraries are at least a ribosomal display library, a bacterial surface library and a proteome. It has been observed that although all expression libraries may be complete, using only one or two expression libraries in an antigen identification programme will not lead to a complete set of antigens due to preferential expression properties of each of the different expression libraries. While it is therefore possible to obtain hyperimmune serum-reactive antigens by using only one or two different expression libraries, this might in many cases not finally result in the identification of a complete set of hyperimmune serum-reactive antigens. Of course, the term "complete" according to the present invention does not indicate a theoretical maximum but is indeed a practical completeness, i.e. that at least 95% of the practically relevant antigens or antigenic determinants have been identified of a given pathogen. The practical relevance is thereby defined by the occurrence of antibodies against given antigens in the patient population.

According to the present invention also serum pools or plasma fractions or other pooled antibody containing body fluids are "plasma pools".

An expression library as used in the present invention should at least allow expression of all potential antigens, e.g. all surface proteins of a given pathogen. With the expression libraries according to the present invention, at least one set of potential antigens of a given pathogen is provided, this set being prefera-

bly the complete theoretical complement of (poly-)peptides encoded by the pathogen's genome (i.e. genomic libraries as described in Example 2) and expressed either in a recombinant host (see Example 3) or in vitro (see Example 4). This set of potential antigens can also be a protein preparation, in the case of extracellular pathogens preferably a protein preparation containing surface proteins of said pathogen obtained from said pathogen grown under defined physiological conditions (see Example 5). While the genomic approach has the potential to contain the complete set of antigens, the latter one has the advantage to contain the proteins in their naturally state i.e. including for instance post-translational modifications or processed forms of these proteins, not obvious from the DNA sequence. These or any other sets of potential antigens from a pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity are hereafter referred to as "expression library". Expression libraries of very different kinds may be applied in the course of the present invention. Suitable examples are given in e.g. Ausubel et al., 1994. Especially preferred are expression libraries representing a display of the genetic set of a pathogen in recombinant form such as in vitro translation techniques, e.g. ribosomal display, or prokaryotic expression systems, e.g. bacterial surface expression libraries or which resemble specific physiological expression states of a given pathogen in a given physiological state, such as a proteome.

Ribosome display is an established method in recombinant DNA technology, which is applicable for each specific pathogen for the sake of the present invention (Schaffitzel et al, 1999). Bacterial surface display libraries will be represented by a recombinant library of a bacterial host displaying a (total) set of expressed peptide sequences of a given pathogen on e.g. a selected outer membrane protein at the bacterial host membrane (Georgiou et al., 1997). Apart from displaying peptide or protein sequences in an outer membrane protein, other bacterial display techniques, such as bacteriophage display technologies and expression via exported proteins are also preferred as bacterial surface expression library (Forrer et al., 1999; Rodi and Makowski, 1993; Georgiou et al., 1997).

The antigen preparation for the first round of screening in the method according to the present invention may be derived from any source containing antibodies to a given pathogen. Preferably, if a plasma pool is used as a source for the antibody preparation, a human plasma pool is selected which comprises donors which had experienced or are experiencing an infection with the given pathogen. Although such a selection of plasma or plasma pools is in principle standard technology in for example the production of hyperimmunoglobulin preparations, it was surprising that such technologies have these effects as especially shown for the preferred embodiments of the present invention.

Preferably the expression libraries are genomic expression libraries of a given pathogen, or alternatively m-RNA, libraries. It is preferred that these genomic or m-RNA libraries are complete genomic or m-RNA expression libraries which means that they contain at least once all possible proteins, peptides or peptide fragments of the given pathogen are expressable. Preferably the genomic expression libraries exhibit a redundancy of at least 2x, more preferred at least 5x, especially at least 10x.

Preferably, the method according to the present invention comprises screening at least a ribosomal display library, a bacterial surface display library and a proteome with the antibody preparation and identifying antigens which bind in at least two, preferably which bind to all, of said screenings to antibodies in said antibody preparation. Such antigens may then be regarded extremely suited as hyperimmunogenic antigens regardless of their way of expression. Preferably the at least two screenings should at least contain the proteome, since the proteome always represents the antigens as naturally expressed proteins including post-translational modifications, processing, etc. which are not obvious from the DNA sequence.

The method according to the present invention may be applied to any given pathogen. Therefore, preferred pathogens are selected from the group of bacterial, viral, fungal and protozoan pathogens. The method according to the present invention is also applicable to cancer, i.e. for the identification of tumor-associated antigens, and for the identification of allergens or

antigens involved in auto-immune diseases. Of course, especially the recombinant methods are rather simple for pathogens having a small genome or a comparatively small number of expressed proteins (such as bacterial or viral pathogens) and are more complicated for complex (eukaryotic) organisms having large genomes. However, also such large genomic libraries of higher organism pathogens may well be analyzed with the method according to the present invention, at least in a faster and more reliable way than with known methods for identifying suitable antigens.

Preferred pathogens to be analyzed or which antigens are to be extracted, respectively, include human immunedeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), Rous sarcoma virus (RSV), Epstein-Barr virus (EBV), influenza virus (IV), rotavirus (RV), Staphylococcus aureus (S.aureus), Staphylococcus epidermidis (S. epidermidis), Chlamydia pneumoniae (C. pneumoniae), Chlamydia trachomatis (C. trachomatis), Mycobacterium tuberculosis (M. tuberculosis), Mycobacterium leprae (M. leprae), Streptococcus pneumoniae (S. pneumoniae), Streptococcus pyogenes (S. pyogenes), Streptococcus agalactiae (S. agalactiae), Enterococcus faecalis (E. faecalis), Bacillus anthracis (B. anthracis), Vibrio cholerae (V. cholerae), Borrelia burgdorferi (B. burgdorferi), Plasmodium sp., fungal diseases such as Pneumocystis carinii, Aspergillus sp., Cryptococcus sp., Candida albicans or parasitic infections such as ascariasis (Ascaris lumbricoides) and taeniasis (Taenia saginata). The method according to the present invention is most applicable for bacteria, worms or candida.

As a model organism for the present application Staphylococcus aureus has been chosen to demonstrate the applicability and efficacy of the method according to the present invention. Especially with respect to the examples it is clear that the invention is easily transferable to all potential pathogens, especially the ones listed above.

It was surprising that the method according to the present invention allows an efficient and fast biological screening of a given pathogen, especially in view of the fact that only a small fraction of a patient's antibody repertoire is directed to a given

pathogen, even in a state where this pathogen is effectively defeated. It has been discovered within the course of the present invention, especially during performance of the S.aureus example that only 1-2% of the antibody repertoire of a patient having high titers against S.aureus are indeed antibodies directed against S.aureus. Moreover, over 70% of this specific 1% portion is directed against non-protein antigens, such as teichoic acid, so that only a total of 0.1% or less of the antibodies are directed to proteinaceous antigens.

One of the advantages of using recombinant expression libraries, especially ribsome display libraries and bacterial surface display libraries, is that the identified hyperimmune serum-reactive antigens may be instantly produced by expression of the coding sequences of the screened and selected clones expressing the hyperimmune serum-reactive antigens without further recombinant DNA technology or cloning steps necessary.

The hyperimmune serum-reactive antigens obtainable by the method according to the present invention may therefore be immediately finished to a pharmaceutical preparation, preferably by addition of a pharmaceutically acceptable carrier and/or excipient, immediately after its production (in the course of the second selection step), e.g. by expression from the expression library platform.

Preferably, the pharmaceutical preparation containing the hyperimmune serum-reactive antigen is a vaccine for preventing or treating an infection with the specific pathogen for which the antigens have been selected.

The pharmaceutical preparation may contain any suitable auxiliary substances, such as buffer substances, stabilisers or further active ingredients, especially ingredients known in connection of vaccine production.

A preferable carrier/or excipient for the hyperimmune serum-reactive antigens according to the present invention is a immunostimulatory compound for further stimulating the immune response to the given hyperimmune serum-reactive antigen. Pref-

erably the immunostimulatory compound in the pharmaceutical preparation according to the present invention is selected from the group of polycationic substances, especially polycationic peptides, immunostimulatory deoxynucleotides, alumn, Freund's complete adjuvans, Freund's incomplete adjuvans, neuroactive compounds, especially human growth hormone, or combinations thereof.

The polycationic compound(s) to be used according to the present invention may be any polycationic compound which shows the characteristic effects according to the WO 97/30721. Preferred polycationic compounds are selected from basic polypeptides, organic polycations, basic polyamino acids or mixtures thereof. These polyamino acids should have a chain length of at least 4 amino acid residues (see: Tuftsin as described in Goldman et al. (1983)). Especially preferred are substances like polylysine, polyarginine and polypeptides containing more than 20%, especially more than 50% of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof. Other preferred polycations and their pharmaceutical compositons are described in WO 97/30721 (e.g. polyethyleneimine) and WO 99/38528. Preferably these polypeptides contain between 20 and 500 amino acid residues, especially between 30 and 200 residues.

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources.

Cationic (poly)peptides may also be anti- microbial with properties as reviewed in Ganz et al, 1999; Hancock, 1999. These (poly)peptides may be of prokaryotic or animal or plant origin or may be produced chemically or recombinantly (Andreu et al., 1998; Ganz et al., 1999; Simmaco et al., 1998). Peptides may also belong to the class of defensins (Ganz, 1999; Ganz et al., 1999). Sequences of such peptides can be, for example, be found in the Antimicrobial Sequences Database under the following internet address:

http://www.bbcm.univ.trieste.it/~tossi/pag2.html

Such host defence peptides or defensives are also a preferred form of the polycationic polymer according to the present inven-

tion. Generally, a compound allowing as an end product activation (or down-regulation) of the adaptive immune system, preferably mediated by APCs (including dendritic cells) is used as polycationic polymer.

Especially preferred for use as polycationic substance in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (International patent application PCT/EP01/09529, incorporated herein by reference), especially antimicrobial peptides derived from mammal cathelicidin, preferably from human, bovine or mouse.

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are cathelin or related or derived substances from cathelin. For example, mouse cathelin is a peptide which has the amino acid sequence NH_2-RLAGLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE-COOH. Related or derived cathelin substances contain the whole or parts of the cathelin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelin molecules. These cathelin molecules are preferred to be combined with the antigen. These cathelin molecules surprisingly have turned out to be also effective as an adjuvant for a antigen without the addition of further adjuvants. It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Another preferred polycationic substance to be used according to the present invention is a synthetic peptide containing at least 2 KLK-motifs separated by a linker of 3 to 7 hydrophobic amino acids (International patent application PCT/EP01/12041, incorporated herein by reference).

Immunostimulatory deoxynucleotides are e.g. neutral or artificial

CpG containing DNA, short stretches of DNA derived from non-vertebrates or in form of short oligonucleotides (ODNs) containing non-methylated cytosine-guanine di-nucleotides (CpG) in a certain base context (e.g. Krieg et al., 1995) but also inosine containing ODNs (I-ODNs) as described in WO 01/93905.

Neuroactive compounds, e.g. combined with polycationic substances are described in WO 01/24822.

According to a preferred embodiment the individual antibody preparation for the second round of screening are derived from patients with have suffered from an acute infection with the given pathogen, especially from patients who show an antibody titer to the given pathogen above a certain minimum level, for example an antibody titer being higher than 80 percentile, preferably higher than 90 percentile, especially higher than 95 percentile of the human (patient or carrier) sera tested. Using such high titer individual antibody preparations in the second screening round allows a very selective identification of the hyperimmune serum-reactive antigens to the given pathogen.

It is important that the second screening with the individual antibody preparations (which may also be the selected serum) allows a selective identification of the hyperimmune serum-reactive antigens from all the promising candidates from the first round. Therefore, preferably at least 10 individual antibody preparations (i.e. antibody preparations (e.g. sera) from at least 10 different individuals having suffered from an infection to the chosen pathogen) should be used in identifying these antigens in the second screening round. Of course, it is possible to use also less than 10 individual preparations, however, selectivity of the step may not be optimal with a low number of individual antibody preparations. On the other hand, if a given hyperimmune serum-reactive antigen (or an antigenic fragment thereof) is recognized in at least 10 individual antibody preparations, preferably at least 30, especially at least 50 individual antibody preparations, identification of hyperimmune serum-reactive antigen is also selective enough for a proper identification. Hyperimmune serum-reactivity may of course be tested with as many individual preparations as possible (e.g. with more than 100 or even with

more than 1000).

Therefore, the relevant portion of the hyperimmune serum-reactive antibody preparation according to the method of the present invention should preferably be at least 10, more preferred at least 30, especially at least 50 individual antibody preparations. Alternatively (or in combination) hyperimmune serum-reactive antigen may preferably be also identified with at least 20%, preferably at least 30%, especially at least 40% of all individual antibody preparations used in the second screening round.

According to a preferred embodiment of the present invention, the sera from which the individual antibody preparations for the second round of screening are prepared (or which are used as antibody preparations), are selected by their titer against the specific pathogen (e.g. against a preparation of this pathogen, such as a lysate, cell wall components and recombinant proteins). Preferably, some are selected with a total IgA titer above 4000 U, especially above 6000 U, and/or an IgG titer above 10 000 U, especially above 12 000 U (U = units, calculated from the OD_{405nm} reading at a given dilution) when whole organism (total lysate or whole cells) is used as antigen in ELISA. Individual proteins with Ig titers of above 800-1000 U are specifically preferred for selecting the hyperimmune serum-reactive antigens according to the present invention only for total titer. The statement for individual proteins can be derived from Fig. 9.

According to the demonstration example which is also a preferred embodiment of the present invention the given pathogen is a Staphylococcus pathogen, especially Staphylococcus aureus and Staphylococcus epidermidis. Staphylococci are opportunistic pathogens which can cause illnesses which range from minor infections to life threatening diseases. Of the large number of Staphylococci at least 3 are commonly associated with human disease: S. aureus, S. epidermidis and rarely S. saprophyticus (Crossley and Archer, 1997). S. aureus has been used within the course of the present invention as an illustrative example of the way the present invention functions. Besides that, it is also an important organism with respect to its severe pathogenic impacts on humans. Staphylococcal infections are imposing an increasing

threat in hospitals worldwide. The appearance and disease causing capacity of Staphylococci are related to the wide-spread use of antibiotics which induced and continue to induce multi-drug resistance. For that reason medical treatment against Staphylococcal infections cannot rely only on antibiotics anymore. Therefore, a tactic change in the treatment of these diseases is desperately needed which aims to prevent infections. Inducing high affinity antibodies of the opsonic and neutralizing type by vaccination helps the innate immune system to eliminate bacteria and toxins. This makes the method according to the present invention an optimal tool for the identification of staphylococcal antigenic proteins.

Every human being is colonized with S. epidermidis. The normal habitats of S. epidermidis are the skin and the mucous membrane. The major habitats of the most pathogenic species, S. aureus, are the anterior nares and perineum. Some individuals become permanent S. aureus carriers, often with the same strain. The carrier stage is clinically relevant because carriers undergoing surgery have more infections than noncarriers. Generally, the established flora of the nose prevents acquisition of new strains. However, colonization with other strains may occur when antibiotic treatment is given that leads to elimination of the susceptible carrier strain. Because this situation occurs in the hospitals, patients may become colonized with resistant nosocomial Staphylococci. These bacteria have an innate adaptability which is complemented by the widespread and sometimes inappropriate use of antimicrobial agents. Therefore hospitals provide a fertile environment for drug resistance to develop (close contact among sick patients, extensive use of antimicrobials, nosocomial infections). Both S. aureus and S. epidermidis have become resistant to many commonly used antibiotics, most importantly to methicillin (MRSA) and vancomycin (VISA). Drug resistance is an increasingly important public health concern, and soon many infections caused by staphylococci may be untreatable by antibiotics. In addition to its adverse effect on public health, antimicrobial resistance contributes to higher health care costs, since treating resistant infections often requires the use of more toxic and more expensive drugs, and can result in longer hospital stays for infected patients.

Moreover, even with the help of effective antibiotics, the most serious staphylococcal infections have 30-50 % mortality.

Staphylococci become potentially pathogenic as soon as the natural balance between microorganisms and the immune system gets disturbed, when natural barriers (skin, mucous membrane) are breached. The coagulase-positive S. aureus is the most pathogenic staphylococcal species, feared by surgeons for a long time. Most frequently it causes surgical wound infections, and induces the formation of abscesses. This local infection might become systemic, causing bacteraemia and sepsis. Especially after viral infections and in elderly, it can cause severe pneumonia. S. aureus is also a frequent cause of infections related to medical devices, such as intravascular and percutan catheters (endocarditis, sepsis, peritonitis), prosthetic devices (septic arthritis, osteomyelitis). S. epidermidis causes diseases mostly related to the presence of foreign body and the use of devices, such as catheter related infections, cerebrospinal fluid shunt infections, peritonitis in dialysed patients (mainly CAPD), endocarditis in individuals with prosthetic valves. This is exemplified in immunocompromised individuals such as oncology patients and premature neonates in whom coagulase-negative staphylococcal infections frequently occur in association with the use of intravascular device. The increase in incidence is related to the increased used of these devices and increasing number of immunocompromised patients.

Much less is known about S. saprophyticus, another coagulase-negative staphylococci, which causes acute urinary tract infection in previously healthy people. With a few exceptions these are women aged 16-25 years.

The pathogenesis of staphylococci is multifactorial. In order to initiate infection the pathogen has to gain access to the cells and tissues of the host, that is adhere. S. aureus expresses-surface proteins that promote attachment to the host proteins such as laminin, fibronectin, elastin, vitronectin, fibrinogen and many other molecules that form part of the extracellular matrix (extracellular matrix binding proteins, ECMBP). S. epider-

midis is equipped with cell surface molecules which promote adherence to foreign material and through that mechanism establish infection in the host. The other powerful weapons staphylococci use are the secreted products, such as enterotoxins, exotoxins, and tissue damaging enzymes. The toxins kill or misguide immune cells which are important in the host defence. The several different types of toxins are responsible for most of the symptoms during infections.

Host defence against S. aureus relies mainly on innate immunological mechanisms. The skin and mucous membranes are formidable barriers against invasion by Staphylococci. However, once the skin or the mucous membranes are breached (wounds, percutan· catheters, etc), the first line of nonadaptive cellular defence begins its co-ordinate action through complement and phagocytes, especially the polymorphonuclear leukocytes (PMNs). These cells can be regarded as the cornerstones in eliminating invading bacteria. As Staphylococci are primarily extracellular pathogens; the major anti-staphylococcal adaptive response comes from the humoral arm of the immune system, and is mediated through three major mechanisms: promotion of opsonization, toxin neutralisation, and inhibition of adherence. It is believed that opsonization is especially important, because of its requirement for an effective phagocytosis. For efficient opsonization the microbial surface has to be coated with antibodies and complement factors for recognition by PMNs through receptors to the Fc fragment of the IgG molecule or to activated C3b. After opsonization, staphylococci are phagocytosed and killed. Moreover, S. aureus can attach to endothelial cells, and be internalised by a phagocytosis-like process. Antibodies bound to specific antigens on the cell surface of bacteria serve as ligands for the attachment to PMNs and promote phagocytosis. The very same antibodies bound to the adhesins and other cell surface proteins are expected to neutralize adhesion and prevent colonization.

There is little clinical evidence that cell mediated immunity has a significant contribution in the defence against Staphylococci, yet one has to admit that the question is not adequately addressed. It is known, however, that Staphylococcus aureus utilizes an extensive array of molecular countermeasures to

manipulate the defensive microenvironment of the infected host by secreting polypeptides referred to as superantigens, which target the multireceptor communication between T-cells and antigen-presenting cells that is fundamental to initiating pathogen-specific immune clearance. Superantigens play a critical role in toxic shock syndrome and food poisoning, yet their function in routine infections is not well understood. Moreover, one cannot expect a long lasting antibody (memory) response without the involvement of T-cells. It is also known that the majority of the anti-staphylococcal antibodies are against T-cell independent antigens (capsular polysacharides, lipoteichoic acid, peptidoglycan) without a memory function. The T-cell dependent proteinaceous antigens can elicit long-term protective antibody responses. These staphylococcal proteins and peptides have not yet been determined.

For all these above mentioned reasons, a tactic change on the war field against staphylococcal infections is badly needed. One way of combating infections is preventing them by active immunisation. Vaccine development against S. aureus has been initiated by several research groups and national institutions worldwide, but there is no effective vaccine approved so far. It has been shown that an antibody deficiency state contributes to staphylococcal persistence, suggesting that anti-staphylococcal antibodies are important in host defence. Antibodies - added as passive immunisation or induced by active vaccination - directed towards surface components could both prevent bacterial adherence, neutralize toxins and promote phagocytosis. A vaccine based on fibronectin binding protein induces protective immunity against mastitis in cattle and suggest that this approach is likely to work in humans (refs). Taking all this together it is suggestive that an effective vaccine should be composed of proteins or polypeptides, which are expressed by all strains and are able to induce high affinity, abundant antibodies against cell surface components of S. aureus. The antibodies should be IgG1 and/or IgG3 for opsonization, and any IgG subtype and IgA for neutralisation of adherence and toxin action. A chemically defined vaccine must be definitely superior compared to a whole cell vaccine (attenuated or killed), since components of S. aureus which paralyze TH cells (superantigens) or inhibit opsonization (protein A)

can be eliminated, and the individual proteins inducing protective antibodies can be selected. Identification of the relevant antigens help to generate effective passive immunisation (humanised monoclonal antibody therapy), which can replace human immunoglobulin administration with all its dangerous side-effects. Neonatal staphylococcal infections, severe septicemia and other life-threatening acute conditions are the primary target of passive immunisation. An effective vaccine offers great potential for patients facing elective surgery in general, and those receiving endovascular devices, in particular. Moreover, patients suffering from chronic diseases which decrease immune responses or undergoing continuous ambulatory peritoneal dialysis are likely to benefit from such a vaccine.

For the illustrative example concerning Staphylococcus aureus three different approaches have been employed in parallel. All three of these methods are based on the interaction of Staphylococcus proteins or peptides with the antibodies present in human sera with the method according to the present invention. This interaction relies on the recognition of epitopes within the proteins which can be short peptides (linear epitopes) or polypeptide domains (structural epitopes). The antigenic proteins are identified by the different methods using pools of pre-selected sera and - in the second screening round - by individual selected sera.

Following the high throughput screening, the selected antigenic proteins are expressed as recombinant proteins or in vitro translated products (in case it can not be expressed in prokaryotic expression systems), and tested in a series of ELISA and Western blotting assays for the assessment of immunogeneicity with a large human serum collection (> 100 uninfected, > 50 patients sera). The preferred antigens are located on the cell surface or secreted, that is accessible extracellularly. Antibodies against the cell wall proteins (such as the Extracellular matrix binding proteins) are expected to serve double purposes: to inhibit adhesion and promote phagocytosis. The antibodies against the secreted proteins are beneficial in toxin neutralisation. It is also known that bacteria communicate with each other through secreted proteins. Neutralizing antibodies against these proteins

will interrupt growth promoting cross-talk between or within staphylococcal species. Bioinformatics (signal sequences, cell wall localisation signals, transmembrane domains) proved to be very useful in assessing cell surface localisation or secretion. The experimental approach includes the isolation of antibodies with the corresponding epitopes and proteins from human serum, and use them as reagents in the following assays: cell surface staining of staphylococci grown under different conditions (FACS, microscopy), determination of neutralizing capacity (toxin, adherence), and promotion of opsonization and phagocytosis (in vitro phagocytosis assay).

The recognition of linear epitopes by antibodies can be based on sequences as short as 4-5 aa. Of course it does not necessarily mean that these short peptides are capable of inducing the given antibody. in vivo. For that reason the defined epitopes, polypeptides and proteins may further be tested in animals (mainly in mice) for their capacity to induce antibodies against the selected proteins in vivo. The antigens with the proven capability to induce antibodies will be tested in animal models for the ability to prevent infections.

The antibodies produced against Staphylococci by the human immune system and present in human sera are indicative of the in vivo expression of the antigenic proteins and their immunogenicity.

Accordingly, novel hyperimmune serum-reactive antigens from Staphylococcus aureus or Staphylococcus epidermidis have been made available by the method according to the present invention. According to another aspect of the present invention the invention relates to a hyperimmune serum-reactive antigen selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3, 4 and 5, especially selected from the group consisting of Seq.ID No. 56, 57, 59, 60, 67, 70, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 85, 87, 88, 89, 90, 92, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 126, 128, 132, 134, 138, 140, 142, 151, 152, 154, 155 and hyperimmune fragments thereof. Accordingly, the present invention also relates to a hyperimmune serum-reactive antigen obtainable by the method according to the present invention

and being selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3, 4 and 5, especially selected from the group consisting of Seq.ID No. 56, 57, 59, 60, 67, 70, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 85, 87, 88, 89, 90, 92, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 126, 128, 132, 134, 138, 140, 142, 151, 152, 154, 155 and hyperimmune fragments thereof.

Antigens from Staphylococcus aureus and Staphylococcus epidermidis have been extracted by the method according to the present invention which may be used in the manufacture of a pharmaceutical preparation, especially for the manufacture of a vaccine against Staphylococcus aureus and Staphylococcus epidermidis infections. Examples of such hyperimmune serum-reactive antigens of Staphylococcus aureus and Staphylococcus epidermidis to be used in a pharmaceutical preparation are selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3, 4 and 5, especially selected from the group consisting of Seq.ID No. 55, 56, 57, 58, 59, 60, 62, 66, 67, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 92, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 126, 128, 130, 132, 134, 138, 140, 142, 151, 152, 154, 155, 158 and hyperimmune fragments thereof for the manufacture of a pharmaceutical preparation, especially for the manufacture of a vaccine against Staphylococcus aureus and Staphylococcus epidermidis infections.

A hyperimmune fragment is defined as a fragment of the identified antigen which is for itself antigenic or may be made antigenic when provided as a hapten. Therefore, also antigen or antigenic fragments showing one or (for longer fragments) only a few amino acid exchanges are enabled with the present invention, provided that the antigenic capacities of such fragments with amino acid exchanges are not severely deteriorated on the exchange(s). i.e. suited for eliciting an appropriate immune response in a individual vaccinated with this antigen and identified by individual antibody preparations from individual sera.

Preferred examples of such hyperimmune fragments of a hyperimmune serum-reactive antigen are selected from the group consisting of

peptides comprising the amino acid sequences of column "predicted immunogenic aa", "Location of identified immunogenic region" and "Serum reactivity with relevant region" of Tables 2a, 2b, 2c and 2d and the amino acid sequences of column "Putative antigenic surface areas" of Table 4 and 5, especially peptides comprising amino acid No. aa 12-29, 34-40, 63-71, 101-110, 114-122, 130-138, 140-195, 197-209, 215-229, 239-253, 255-274 and 39-94 of Seq.ID No. 55,
aa 5-39, 111-117, 125-132, 134-141, 167-191, 196-202, 214-232, 236-241, 244-249, 292-297, 319-328, 336-341, 365-380, 385-391, 407-416, 420-429, 435-441, 452-461, 477-488, 491-498, 518-532, 545-556, 569-576, 581-587, 595-602, 604-609, 617-640, 643-651, 702-715, 723-731, 786-793, 805-811, 826-839, 874-889, 37-49, 63-77 and 274-334, of Seq.ID No.56,
aa 28-55, 82-100, 105-111, 125-131, 137-143, 1-49, of Seq.ID No. 57,
aa 33-43, 45-51, 57-63, 65-72, 80-96, 99-110, 123-129, 161-171, 173-179, 185-191, 193-200, 208-224, 227-246, 252-258, 294-308, 321-329, 344-352, 691-707, 358-411 and 588-606, of Seq.ID No. 58,
aa 16-38, 71-77, 87-94, 105-112, 124-144, 158-164, 169-177, 180-186, 194-204, 221-228, 236-245, 250-267, 336-343, 363-378, 385-394, 406-412, 423-440, 443-449, 401-494, of Seq.ID No. 59,
aa 18-23, 42-55, 69-77, 85-98, 129-136, 182-188, 214-220, 229-235, 242-248, 251-258, 281-292, 309-316, 333-343, 348-354, 361-367, 393-407, 441-447, 481-488, 493-505, 510-515, 517-527, 530-535, 540-549, 564-583, 593-599, 608-621, 636-645, 656-670, 674-687, 697-708, 726-734, 755-760, 765-772, 785-792, 798-815, 819-824, 826-838, 846-852, 889-904, 907-913, 932-939, 956-964, 982-1000, 1008-1015, 1017-1024, 1028-1034, 1059-1065, 1078-1084, 1122-1129, 1134-1143, 1180-1186, 1188-1194, 1205-1215, 1224-1230, 1276-1283, 1333-1339, 1377-1382, 1415-1421, 1448-1459, 1467-1472, 1537-1545, 1556-1566, 1647-1654, 1666-1675, 1683-1689, 1722-1737, 1740-1754, 1756-1762, 1764-1773, 1775-1783, 1800-1809, 1811-1819, 1839-1851, 1859-1866, 1876-1882, 1930-1939, 1947-1954, 1978-1985, 1999-2007, 2015-2029, 2080-2086, 2094-2100, 2112-2118, 2196-2205, 2232-2243, 198-258, 646-727 and 2104-2206, of Seq.ID No. 60,
aa 10-29, 46-56, 63-74, 83-105, 107-114, 138-145, 170-184, 186-193, 216-221, 242-248, 277-289, 303-311, 346-360, 379-389, 422-428, 446-453, 459-469, 479-489, 496-501, 83-156, of Seq.ID No. 62,

aa 14-22, 32-40, 52-58, 61-77, 81-93, 111-117, 124-138, 151-190, 193-214, 224-244, 253-277, 287-295, 307-324, 326-332, 348-355, 357-362, 384-394, 397-434, 437-460, 489-496, 503-510, 516-522, 528-539, 541-547, 552-558, 563-573, 589-595, 602-624, 626-632, 651-667, 673-689, 694-706, 712-739, 756-790, 403-462, of Seq.ID No. 66,

aa 49-56, 62-68, 83-89, 92-98, 109-115, 124-131, 142-159, 161-167, 169-175, 177-188, 196-224, 230-243, 246-252, 34-46, of Seq.ID No. 67,

aa 11-20, 26-47, 69-75, 84-92, 102-109, 119-136, 139-147, 160-170, 178-185, 190-196, 208-215, 225-233, 245-250, 265-272, 277-284, 300-306, 346-357, 373-379, 384-390, 429-435, 471-481, 502-507, 536-561, 663-688, 791-816, 905-910, 919-933, 977-985, 1001-1010, 1052-1057, 1070-1077, 1082-1087, 1094-1112, 493-587, 633-715 and 704-760, of Seq.ID No.70,

aa.6-20, 53-63, 83-90, 135-146, 195-208, 244-259, 263-314, 319-327, 337-349, 353-362, 365-374, 380-390, 397-405, 407-415, 208-287 and 286-314, of Seq.ID No. 71,

aa 10-26, 31-43, 46-58, 61-66, 69-79, 85-92, 100-115, 120-126, 128-135, 149-155, 167-173, 178-187, 189-196, 202-222, 225-231, 233-240, 245-251, 257-263, 271-292, 314-322, 325-334, 339-345, 59-74, of Seq.ID No. 72,

aa 4-9, 15-26, 65-76, 108-115, 119-128, 144-153, 38-52 and 66-114, of Seq.ID No. 73,

aa 5-22, 42-50, 74-81, 139-145, 167-178, 220-230, 246-253, 255-264, 137-237 and 250-267, of Seq.ID No. 74,

aa 10-26, 31-44, 60-66, 99-104, 146-153, 163-169, 197-205, 216-223, 226-238, 241-258, 271-280, 295-315, 346-351, 371-385, 396-407, 440-446, 452-457, 460-466, 492-510, 537-543, 546-551, 565-582, 590-595, 635-650, 672-678, 686-701, 705-712, 714-721, 725-731, 762-768, 800-805, 672-727, of Seq.ID No. 75,

aa 5-32, 35-48, 55-76, of Seq.ID No. 76,

aa 7-35, 54-59, 247-261, 263-272, 302-320, 330-339, 368-374, 382-411, 126-143 and 168-186, of Seq.ID No. 77,

aa 5-24, 88-94, 102-113, 132-143, 163-173, 216-224, 254-269, 273-278, 305-313, 321-327, 334-341, 31-61 and 58-74, of Seq.ID No. 78,

aa 16-24, 32-39, 43-49, 64-71, 93-99, 126-141, 144-156, 210-218, 226-233, 265-273, 276-284, 158-220, of Seq.ID No. 79,

aa 49-72, 76-83, 95-105, 135-146, 148-164, 183-205, 57-128, of

Seq.ID No. 80,
aa 6-15, 22-32, 58-73, 82-88, 97-109, 120-131, 134-140, 151-163, 179-185, 219-230, 242-255, 271-277, 288-293, 305-319, 345-356, 368-381, 397-406, 408-420, 427-437, 448-454, 473-482, 498-505, 529-535, 550-563, 573-580, 582-590, 600-605, 618-627, 677-685, 718-725, 729-735, 744-759, 773-784, 789-794, 820-837, 902-908, 916-921, 929-935, 949-955, 1001-1008, 1026-1032, 1074-1083, 1088-1094, 1108-1117, 1137-1142, 1159-1177, 1183-1194, 1214-1220, 1236-1252, 1261-1269, 1289-1294, 1311-1329, 1336-1341, 1406-1413, 1419-1432, 1437-1457, 1464-1503, 1519-1525, 1531-1537, 1539-1557, 1560-1567, 1611-1618, 1620-1629, 1697-1704, 1712-1719, 1726-1736, 1781-1786, 1797-1817, 1848-1854, 1879-1890, 1919-1925, 1946-1953, 1974-1979, 5 to 134, of Seq.ID No. 81,
aa 6-33, 40-46, 51-59, 61-77, 84-104, 112-118, 124-187, 194-248, 252-296, 308-325, 327-361, 367-393, 396-437, 452-479, 484-520, 535-545, 558-574, 582-614, 627-633, 656-663, 671-678, 698-704, 713-722, 725-742, 744-755, 770-784, 786-800, 816-822, 827-837, 483-511, of Seq.ID No. 82,
aa 4-19, 57-70, 79-88, 126-132, 144-159, 161-167, 180-198, 200-212, 233-240, 248-255, 276-286, 298-304, 309-323, 332-346, 357-366, 374-391, 394-406, 450-456, 466-473, 479-487, 498-505, 507-519, 521-530, 532-540, 555-565, 571-581, 600-611, 619-625, 634-642, 650-656, 658-665, 676-682, 690-699, 724-733, 740-771, 774-784, 791-797, 808-815, 821-828, 832-838, 876-881, 893-906, 922-929, 938-943, 948-953, 969-976, 1002-1008, 1015-1035, 1056-1069, 1105-1116, 1124-1135, 1144-1151, 1173-1181, 1186-1191, 1206-1215, 1225-1230, 1235-1242, 6-66, 65-124 and 590-604, of Seq.ID No. 83,
aa 5-32, 66-72, 87-98, 104-112, 116-124, 128-137, 162-168, 174-183, 248-254, 261-266, 289-303, 312-331, 174-249, of Seq.ID No. 84,
aa 4-21, 28-40, 45-52, 59-71, 92-107, 123-137, 159-174, 190-202, 220-229, 232-241, 282-296, 302-308, 312-331, 21-118, of Seq.ID No. 85,
aa 9-28, 43-48, 56-75, 109-126, 128-141, 143-162, 164-195, 197-216, 234-242, 244-251, 168-181, of Seq.ID No. 87,
aa 4-10, 20-42, 50-86, 88-98, 102-171, 176-182, 189-221, 223-244, 246-268, 276-284, 296-329, 112-188, of Seq.ID No. 88,
aa 4-9, 13-24, 26-34, 37-43, 45-51, 59-73, 90-96, 99-113, 160-173, 178-184, 218-228, 233-238, 255-262, 45-105, 103-166 and 66-153, of Seq.ID No. 89,

aa 13-27, 42-63, 107-191, 198-215, 218-225, 233-250, 474-367, of Seq.ID No. 90;
aa 26-53, 95-123, 164-176, 189-199, 8-48, of Seq.ID No. 92,
aa 7-13, 15-23, 26-33, 68-81, 84-90, 106-117, 129-137, 140-159, 165-172, 177-230, 234-240, 258-278, 295-319, 22-56, 23-99, 97-115, 233-250 and 245-265, of Seq.ID No. 94,
aa 13-36, 40-49, 111-118, 134-140, 159-164, 173-183, 208-220, 232-241, 245-254, 262-271, 280-286, 295-301, 303-310, 319-324, 332-339, 1-85, 54-121 and 103-185, of Seq.ID No. 95,
aa 39-44, 46-80, 92-98, 105-113, 118-123, 133-165, 176-208, 226-238, 240-255, 279-285, 298-330, 338-345, 350-357, 365-372, 397-402, 409-415, 465-473, 488-515, 517-535, 542-550, 554-590, 593-601, 603-620, 627-653, 660-665, 674-687, 698-718, 726-739, 386-402, of Seq.ID No. 96,
aa 5-32, 34-49, 1-43, of Seq.ID No. 97,
aa 10-27, 37-56, 64-99, 106-119, 121-136, 139-145, 148-178, 190-216, 225-249, 251-276, 292-297, 312-321, 332-399, 403-458, 183-200, of Seq.ID No. 99,
aa 5-12, 15-20, 43-49, 94-106, 110-116, 119-128, 153-163, 175-180, 185-191, 198-209, 244-252, 254-264, 266-273, 280-288, 290-297, 63-126, of Seq.ID No. 100,
aa 5-44, 47-55, 62-68, 70-78, 93-100, 128-151, 166-171, 176-308, 1-59, of Seq.ID No. 101,
aa 18-28, 36-49, 56-62, 67-84, 86-95, 102-153, 180-195, 198-218, 254-280, 284-296, 301-325, 327-348, 353-390, 397-402, 407-414, 431-455, 328-394, of Seq.ID No. 102,
aa 7-37, 56-71, 74-150, 155-162, 183-203, 211-222, 224-234, 242-272, 77-128, of Seq.ID No. 103,
aa 34-58, 63-69, 74-86, 92-101, 130-138, 142-150, 158-191, 199-207, 210-221, 234-249, 252-271, 5-48, of Seq.ID No. 104,
aa 12-36, 43-50, 58-65, 73-78, 80-87, 108-139, 147-153, 159-172, 190-203, 211-216, 224-232, 234-246, 256-261, 273-279, 286-293, 299-306, 340-346, 354-366, 167-181, of Seq.ID No. 106,
aa 61-75, 82-87, 97-104, 113-123, 128-133, 203-216, 224-229, 236-246, 251-258, 271-286, 288-294, 301-310, 316-329, 337-346, 348-371, 394-406, 418-435, 440-452 of Seq.ID No. 112,
aa 30-37, 44-55, 83-91, 101-118, 121-128, 136-149, 175-183, 185-193, 206-212, 222-229, 235-242 of Seq.ID No. 114,
aa 28-38, 76-91, 102-109, 118-141, 146-153, 155-161, 165-179, 186-202, 215-221, 234-249, 262-269, 276-282, 289-302, 306-314,

321-326, 338-345, 360-369, 385-391 of Seq.ID No. 116,
aa 9-33, 56-62,75-84, 99-105, 122-127, 163-180, 186-192, 206-228, 233-240, 254-262, 275-283, 289-296, 322-330, 348-355, 416-424, 426-438, 441-452, 484-491, 522-528, 541-549, 563-569, 578-584, 624-641, 527-544, of Seq.ID No. 142,
aa 37-42, 57-62, 121-135, 139-145, 183-190, 204-212, 220-227, 242-248, 278-288, 295-30, 304-309, 335-341, 396-404, 412-433, 443-449, 497-503, 505-513, 539-545, 552-558, 601-617, 629-649, 702-711, 736-745, 793-804, 814-829, 843-858, 864-885, 889-895, 905-913, 919-929, 937-943, 957-965, 970-986, 990-1030, 1038-1049, 1063-1072, 1080-1091, 1093-1116, 1126-1136, 1145-1157, 1163-1171, 1177-1183, 1189-1196, 1211-1218, 1225-1235, 1242-1256, 1261-1269, 624-684, of Seq.ID No. 151,
aa 8-23, 31-38, 42-49, 61-77, 83-90, 99-108, 110-119, 140-147, 149-155, 159-171, 180-185, 189-209, 228-234, 245-262, 264-275, 280-302, 304-330, 343-360, 391-409, 432-437, 454-463, 467-474, 478-485, 515-528, 532-539, 553-567, 569-581, 586-592, 605-612, 627-635, 639-656, 671-682, 700-714, 731-747, 754-770, 775-791, 797-834, 838-848, 872-891, 927-933, 935-942, 948-968, 976-986, 1000-1007, 1029-1037, 630-700, of Seq.ID No. 152,
aa 17-25, 27-55, 84-90, 95-101, 115-121, 55-101, of Seq.ID No. 154,
aa 13-28, 40-46, 69-75, 86-92, 114-120, 126-137, 155-172, 182-193, 199-206, 213-221, 232-238, 243-253, 270-276, 284-290, 22-100, of Seq.ID No. 155 and
aa 7-19, 46-57, 85-91, 110-117, 125-133, 140-149, 156-163, 198-204, 236-251, 269-275, 283-290, 318-323, 347-363, 9-42 and 158-174, of Seq.ID No. 158,
aa 7-14, 21-30, 34-50, 52-63, 65-72, 77-84, 109-124, 129-152, 158-163, 175-190, 193-216, 219-234 of Seq.ID.No. 168,
aa 5-24, 38-44, 100-106, 118-130, 144-154, 204-210, 218-223, 228-243, 257-264, 266-286, 292-299 of Seq.ID.No. 174,
aa 29-44, 74-83, 105-113, 119-125, 130-148, 155-175, 182-190, 198-211, 238-245 of Seq.ID.No. 176, and fragments comprising at least 6, preferably more than 8, especially more than 10 aa of said sequences . All these fragments individually and each independently form a preferred selected aspect of the present invention.

Especially suited helper epitopes may also be derived from these

antigens. Especially preferred helper epitopes are peptides comprising fragments selected from the peptides mentioned in column "Putative antigenic surface areas" in Tables 4 and 5 and from the group of aa 6-40, 583-598, 620-646 and 871-896 of Seq.ID.No.56, aa 24-53 of Seq.ID.No.70, aa 240-260 of Seq.ID.No.74, aa 1660-1682 and 1746-1790 of Seq.ID.No. 81, aa 1-29, 680-709, and 878-902 of Seq.ID.No. 83, aa 96-136 of Seq.ID.No. 89, aa 1-29, 226-269 and 275-326 of Seq.ID.No. 94, aa 23-47 and 107-156 of Seq.ID.No. 114 and aa 24-53 of Seq.ID.No. 142 and fragments thereof being T-cell epitopes.

According to another aspect, the present invention relates to a vaccine comprising such a hyperimmune serum-reactive antigen or a fragment thereof as identified above for Staphylococcus aureus and Staphylococcus epidermidis. Such a vaccine may comprise one or more antigens against S. aureus or S. epidermidis. Optionally, such S. aureus or S. epidermidis antigens may also be combined with antigens against other pathogens in a combination vaccine. Preferably this vaccine further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, especially polycationic peptides, immunostimulatory deoxynucleotides (ODNs), neuroactive compounds, especially human growth hormone, alumn, Freund's complete or incomplete adjuvans or combinations thereof. Such a vaccine may also comprise the antigen displayed on a surface display protein platform on the surface of a genetically engineered microorganism such as E. coli.

According to another aspect, the present invention relates to specific preparations comprising antibodies raised against at least one of the Staphylococcus aureus and Staphylococcus epidermidis antigens or Staphylococcus aureus and Staphylococcus epidermidis antigen fragments as defined above. These antibodies are preferably monoclonal antibodies.

Methods for producing such antibody preparations, polyclonal or monoclonal, are well available to the man skilled in the art and properly described in the prior art. A preferred method for producing such monoclonal antibody preparation is characterized by the following steps

- initiating an immune response in a non human animal by administering a Staphylococcus antigen or a fragment thereof, as defined above, to said animal,
- removing the spleen or spleen cells from said animal,
- producing hybridoma cells of said spleen or spleen cells,
- selecting and cloning hybridoma cells specific for said antigen and
- producing the antibody preparation by cultivation of said cloned hybridoma cells and optionally further purification steps.

Preferably, removing of the spleen or spleen cells is connected with killing said animal.

Monoclonal antibodies and fragments thereof can be chimerized or humanized (Graziano et al. 1995) to enable repeated administration. Alternatively human monoclonal antibodies and fragments thereof can be obtained from phage-display libraries (McGuinnes et al., 1996) or from transgenic animals (Brüggemann et al., 1996).

A preferred method for producing polyclonal antibody preparations to said Staphylococcus aureus or Staphylococcus epidermidis antigens identified with the present invention is characterized by the following steps

- initiating an immune response in a non human animal by administering a Staphylococcus antigen or a fragment thereof, as defined above, to said animal,
- removing an antibody containing body fluid from said animal,
- and
- producing the antibody preparation by subjecting said antibody containing body fluid to further purification steps.

These monoclonal or polyclonal antibody preparations may be used for the manufacture of a medicament for treating or preventing diseases due to staphylococcal infection. Moreover, they may be used for the diagnostic and imaging purposes.

The method is further described in the following examples and in the figures, but should not be restricted thereto.

Figure 1 shows the pre-selection of sera based on anti-staphylococcal antibody titers measured by ELISA.

Figure 2 shows the size distribution of DNA fragments in the LSA50/6 library in pMAL4.1.

Figure 3 shows the MACS selection with biotinylated human serum. The LSA50/6 library in pMAL9.1 was screened with 10 µg biotinylated, human serum in the first (A) and with 1 µg in the second selection round (B). P.serum, patient serum; B.serum, infant serum. Number of cells selected after the 2nd and 3rd elution are shown for each selection round.

Figure 4 shows the serum reactivity with specific clones isolated by bacterial surface display as analyzed by Western blot analysis with patient serum at a dilution of 1 : 5000.

Figure 5 shows peptide ELISA with serum from patients and healthy individuals with an epitope identified by ribosome display.

Figure 6 shows representative 2D Immunoblot of S. aureus surface proteins detected with human sera. 800 µg protein from S. aureus/COL grown on BHI were resolved by IEF (pI 4-7) and SDS-PAGE (9-16%), and subsequently transferred to PVDF membrane. After blocking, the membrane was incubated with sera IC35 (1:20,000). Binding of serum IgG was visualized by an anti-human IgG/HRPO conjugate and ECL development.

Figure 7 demonstrates a representative 2D gel showing S. aureus surface proteins stained by Coomassie Blue. 1 mg protein from S. aureus/COL were resolved by IEF (pI 4-7) and SDS-PAGE (9-16%). Spots selected for sequencing after serological proteome analysis are marked.

Figures 8Aand 8B show the structure of LPXTG cell wall proteins.

Figure 9 shows the IgG response in uninfected (N, C) and infected (P) patients to LPXTGV, a novel antigen and probable surface adhesin of S. aureus, discovered by both the inventive bacterial

surface-display and proteomics approaches.

Figure 10 shows the surface staining of S. aureus with purified anti-LPXTGV IgGs.

Figure 11 shows a 2D gel where S. aureus surface proteins are stained by Coomassie Blue (left). 1 mg protein from S. aureus/agr grown to early log phase was resolved by IEF (pI 6-11) and SDS-PAGE (9-16%). Spots selected for sequencing after serological proteome analysis are marked. Corresponding 2D-immunoblot (right). 800 µg protein from the same preparation was resolved in parallel by 2DE, and subsequently transferred to PVDF membrane. After blocking, the membrane was incubated with the P-pool (1:10,000). Binding of serum IgG was visualized by an anti-human IgG/HRPO conjugate and ECL development.

E X A M P L E S

Discovery of novel Staphyloccocus aureus antigens

Example 1: Preparation of antibodies from human serum

The antibodies produced against staphylococci by the human immune system and present in human sera are indicative of the in vivo expression of the antigenic proteins and their immunogenicity. These molecules are essential for the identification of individual antigens in the approach as the present invention which is based on the interaction of the specific anti-staphylococcal antibodies and the corresponding S. aureus peptides or proteins. To gain access to relevant antibody repertoires, human sera were collected from I. patients with acute S. aureus infections, such as bacteriaemia, sepsis, infections of intravascular and percutan catheters and devices, wound infections, and superficial and deep soft tissue infection. S. aureus was shown to be the causative agent by medical microbiological tests. II. A collection of serum samples from uninfected adults was also included in the present analysis, since staphylococcal infections are common, and antibodies are present as a consequence of natural immunization from

previous encounters with Staphylococci from skin and soft tissue infections (furunculus, wound infection, periodontitits etc.).

The sera were characterized for S. aureus antibodies by a series of ELISA assays. Several styaphylococcal antigens have been used to prove that the titer measured was not a result of the sum of cross-reactive antibodies. For that purpose not only whole cell S. aureus (protein A deficient) extracts (grown under different conditions) or whole bacteria were used in the ELISA assays, but also individual cell wall components, such as lipoteichoic acid and peptidoglycan isolated from S. aureus. More importantly, a recombinant protein collection was established representing known staphylococcal cell surface proteins for the better characterization of the present human sera collections.

Recently it was reported that not only IgG, but also IgA serum antibodies can be recognized by the FcRIII receptors of PMNs and promote opsonization (Phillips-Quagliata et al., 2000; Shibuya et al., 2000). The primary role of IgA antibodies is neutralization, mainly at the mucosal surface. The level of serum IgA reflects the quality, quantity and specificity of the dimeric secretory IgA. For that reason the serum collection was not only analyzed for anti-staphylococcal IgG, but also for IgA levels. In the ELISA assays highly specific secondary reagents were used to detect antibodies from the high affinity types, such as IgG and IgA, and avoided IgM. Production of IgM antibodies occurs during the primary adaptive humoral response, and results in low affinity antibodies, while IgG and IgA antibodies had already undergone affinity maturation, and are more valuable in fighting or preventing disease

Experimental procedures

Enzyme linked immune assay (ELISA). ELISA plates were coated with 2-10 µg/ml of the different antigens in coating buffer (sodium carbonate pH 9.2). Serial dilutions of sera (100-100.000) were made in TBS-BSA. Highly specific (cross-adsorbed) HRP (Horse Radish Peroxidase)-labeled anti-human IgG or anti-human IgA secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (~ 2.000x). Antigen-antibody complexes were quantified by measuring the conversion of the sub-

strate (ABTS) to colored product based on OD_{405nm} readings in an automated ELISA reader (Wallace Victor 1420). The titers were compared at given dilution where the dilution response was linear (Table 1). The ~ 100 sera were ranked based on the reactivity against multiple staphylococcal components, and the highest ones (above 90 percentile) were selected for further analysis in antigen identification. Importantly, the anti-staphylococcal antibodies from sera of clinically healthy individuals proved to be very stable, giving the same high ELISA titers against all the staphylococcal antigens measured after 3, 6 and 9 months (data not shown). In contrast, anti-S. aureus antibodies in patients decrease, then disappear after a couple of weeks following the infection (Coloque-Navarro et al, 1998). However, antibodies from patients are very important, since these are direct proof of the in vivo expression of the bacterial antigens tested in or ELISAs or identified as immunogenic during the screens according to the present invention.

This comprehensive approach followed during antibody characterization is unique, and led to unambiguous identification of anti-staphylococcal hyperimmune sera.

Purification of antibodies for genomic screening. Five sera from both the patient and the noninfected group were selected based on the overall anti-staphylococcal titers. Antibodies against E. coli proteins were removed by either incubating the heat inactivated sera with whole cell E. coli (DH5a, transformed with pHIE11, grown under the same condition as used for bacterial display) or with E. coli lysate affinity chromatography for ribosome display. Highly enriched preparations of IgG from the pooled, depleted sera were generated by protein G affinity chromatography, according to the manufacturer's instructions (UltraLink Immobilized Protein G, Pierce). IgA antibodies were purified also by affinity chromatography using biotin-labeled anti-human IgA (Southern Biotech) immobilized on Streptavidin-agarose (GIBCO BRL). The efficiency of depletion and purification was checked by SDS-PAGE, Western blotting, ELISA, and protein concentration measurements. For proteomics, the depletion the IgG and IgA preparation was not necessary, since the secondary reagent ensured the specificity.

Example 2: Generation of highly random, frame-selected, small-fragment, genomic DNA libraries of Staphylococcus aureus

Experimental procedures

Preparation of staphylococcal genomic DNA. This method was developed as a modification of two previously published protocols (Sohail, 1998, Betley et al., 1984) and originally specifically adapted for the methicillin resistant Staphylococcus aureus strain COL to obtain genomic DNA in high quality and large scale. 500 ml BHI (Brain Heart Infusion) medium supplemented with 5 µg/ml Tetracycline was inoculated with bacteria from a frozen stab and grown with aeration and shaking for 18 h at 37°. The culture was then harvested in two aliquots of 250 ml each, centrifuged with 1600 x g for 15 min and the supernatant was removed. Bacterial pellets were carefully re-suspended in 26 ml of 0.1 mM Tris-HCl, pH 7.6 and centrifuged again with 1600 x g for 15 min. Pellets were re-suspended in 20 ml of 1 mM Tris-HCl, pH 7.6, 0.1 mM EDTA and transferred into sterile 50 ml polypropylene tubes. 1 ml of 10 mg/ml heat treated RNase A and 200 U of RNase T1 were added to each tube and the solution mixed carefully. 250 µl of Lysostaphin (10 mg/ml stock, freshly prepared in ddH_2O) was then added to the tubes, mixed thoroughly and incubated at 40°C for 10 min in a shaking water bath under continuous agitation. After the addition of 1 ml 10 % SDS, 40 µl of Proteinase K (25 mg/ml stock) and 100 µl of Pronase (10 mg/ml), tubes were again inverted several times and incubated at 40°C for 5 min in a shaking water bath. 3.75 ml of 5 M NaCl and 2.5 ml of cetyl trimethyl-ammonium bromide solution (CTAB) (10% w/v, 4% w/v NaCl) were then added and tubes were further incubated at 65°C in a shaking water bath for 10 min. Samples were cooled to room temperature and extracted with PhOH/$CHCl_3$/IAA (25:24:1) and with $CHCl_3$/IAA (24:1). Aqueous phases were carefully collected and transferred to new sterile 50-ml tubes. To each tube 1.5 ml of Strataclean™ Resin was added, mixed gently but thoroughly and incubated for one minute at room temperature. Samples were centrifuged and the upper layers containing the DNA were collected into clean 50ml-tubes. DNA was precipitated at room temperature by adding 0.6 x volume of Isopropanol, spooled from the solution with a sterile Pasteur pipette and transferred into tubes con-

taining 80% ice cold ethanol. DNA was recovered by centrifuging the precipitates with 10-12 000 x g, then dried on air and dissolved in ddH_2O.

Preparation of small genomic DNA fragments. Genomic DNA fragments were mechanically sheared into fragments ranging in size between 150 and 300 bp using a cup-horn sonicator (Bandelin Sonoplus UV 2200 sonicator equipped with a BB5 cup horn, 10 sec. pulses at 100 % power output) or into fragments of size between 50 and 70 bp by mild DNase I treatment (Novagen). It was observed that sonication yielded a much tighter fragment size distribution when breaking the DNA into fragments of the 150-300 bp size range. However, despite extensive exposure of the DNA to ultrasonic wave-induced hydromechanical shearing force, subsequent decrease in fragment size could not be efficiently and reproducibly achieved. Therefore, fragments of 50 to 70 bp in size were obtained by mild DNase I treatment using Novagen's shotgun cleavage kit. A 1:20 dilution of DNase I provided with the kit was prepared and the digestion was performed in the presence of $MnCl_2$ in a 60 µl volume at 20°C for 5 min to ensure double-stranded cleavage by the enzyme. Reactions were stopped with 2 µl of 0.5 M EDTA and the fragmentation efficiency was evaluated on a 2% TAE-agarose gel. This treatment resulted in total fragmentation of genomic DNA into near 50-70 bp fragments. Fragments were then blunt-ended twice using T4 DNA Polymerase in the presence of 100 µM each of dNTPs to ensure efficient flushing of the ends. Fragments were used immediately in ligation reactions or frozen at -20°C for subsequent use.

Description of the vectors. The vector pMAL4.1 was constructed on a pEH1 backbone (Hashemzadeh-Bonehi et al., 1998) with the Kanamycin resistance gene. In addition it harbors a b-lactamase (bla) gene cloned into the multiple cloning site. The bla gene is preceded by the leader peptide sequence of ompA to ensure efficient secretion across the cytoplasmic membrane. A Sma I restriction site serves for library insertion. The Sma I site is flanked by an upstream FseI site and a downstream NotI site which were used for recovery of the selected fragments. The three restriction sites are inserted after the ompA leader sequence in such a way that the bla gene is transcribed in the -1 reading frame result-

ing in a stop codon 15 bp after the NotI site. A +1 bp insertion restores the bla ORF so that b-lactamase protein is produced with a consequent gain of Ampicillin resistance.

The vector pMAL4.31 was constructed on a pASK-IBA backbone (Skerra, 1994) with the b-lactamase gene exchanged with the Kanamycin resistance gene. In addition it harbors a b-lactamase (bla) gene cloned into the multiple cloning site. The sequence encoding mature b-lactamase is preceded by the leader peptide sequence of ompA to allow efficient secretion across the cytoplasmic membrane. Furthermore a sequence encoding the first 12 amino acids (spacer sequence) of mature b-lactamase follows the ompA leader peptide sequence to avoid fusion of sequences immediately after the leader peptidase cleavage site, since e.g. clusters of positive charged amino acids in this region would decrease or abolish translocation across the cytoplasmic membrane (Kajava et al., 2000). A SmaI restriction site serves for library insertion. The SmaI site is flanked by an upstream FseI site and a downstream NotI site which were used for recovery of the selected fragment. The three restriction sites are inserted after the sequence encoding the 12 amino acid spacer sequence in such a way that the bla gene is transcribed in the -1 reading frame resulting in a stop codon 15 bp after the NotI site. A +1 bp insertion restores the bla ORF so that b-lactamase protein is produced with a consequent gain of Ampicillin resistance.

The vector pMAL9.1 was constructed by cloning the lamB gene into the multiple cloning site of pEH1. Subsequently, a sequence was inserted in lamB after amino acid 154, containing the restriction sites FseI, SmaI and NotI. The reading frame for this insertion was chosen in a way that transfer of frame-selected DNA fragments excised by digestion with FseI and NotI from plasmids pMAL4.1 or pMAL4.31 to plasmid pMAL9.1 will yield a continuous reading frame of lamB and the respective insert.

The vector pHIE11 was constructed by cloning the fhuA gene into the multiple cloning site of pEH1. Thereafter, a sequence was inserted in fhuA after amino acid 405, containing the restriction site FseI, XbaI and NotI. The reading frame for this insertion was chosen in a way that transfer of frame-selected DNA fragments excised by digestion with FseI and NotI from plasmids pMAL4.1 or

pMAL4.31 to plasmid pHIE11 will yield a continuous reading frame of fhuA and the respective insert.

Cloning and evaluation of the library for frame selection. Genomic S. aureus DNA fragments were ligated into the SmaI site of either the vector pMAL4.1 or pMAL4.31. Recombinant DNA was electroporated into DH10B electrocompetent E. coli cells (GIBCO BRL) and transformants plated on LB-agar supplemented with Kanamycin (50 µg/ml) and Ampicillin (50 µg/ml). Plates were incubated over night at 37°C and colonies collected for large scale DNA extraction. A representative plate was stored and saved for collecting colonies for colony PCR analysis and large-scale sequencing. A simple colony PCR assay was used to initially determine the rough fragment size distribution as well as insertion efficiency. From sequencing data the precise fragment size was evaluated, junction intactness at the insertion site as well as the frame selection accuracy (3n+1 rule).

Cloning and evaluation of the library for bacterial surface display. Genomic DNA fragments were excised from the pMAL4.1 or pMAL4.31 vector, containing the S. aureus library with the restriction enzymes FseI and NotI. The entire population of fragments was then transferred into plasmids pMAL9.1 (LamB) or pHIE11 (FhuA) which have been digested with FseI and NotI. Using these two restriction enzymes, which recognise an 8 bp GC rich sequence, the reading frame that was selected in the pMAL4.1 or pMAL4.31 vector is maintained in each of the platform vectors. The plasmid library was then transformed into E. coli DH5a cells by electroporation. Cells were plated onto large LB-agar plates supplemented with 50 µg/ml Kanamycin and grown over night at 37°C at a density yielding clearly visible single colonies. Cells were then scraped off the surface of these plates, washed with fresh LB medium and stored in aliquots for library screening at -80°C.

Results

Libraries for frame selection. Two libraries (LSA50/6 and LSA250/1) were generated in the pMAL4.1 vector with sizes of approximately 50 and 250 bp, respectively. For both libraries a total number of clones after frame selection of 1-2x 10^6 was

received using approximately 1 µg of pMAL4.1 plasmid DNA and 50 ng of fragmented genomic S. aureus DNA. To assess the randomness of the LSA50/6 library, 672 randomly chosen clones were sequenced. The bioinformatic analysis showed that of these clones none was present more than once. Furthermore, it was shown that 90% of the clones fell in the size range of 19 to 70 bp with an average size of 25 bp (Figure 2). All 672 sequences followed the 3n+1 rule, showing that all clones were properly frame selected.

Bacterial surface display libraries. The display of peptides on the surface of E. coli required the transfer of the inserts from the LSA50/6 library from the frame selection vector pMAL4.1 to the display plasmids pMAL9.1 (LamB) or pHIE11 (FhuA). Genomic DNA fragments were excised by FseI and NotI restriction and ligation of 5ng inserts with 0.1µg plasmid DNA resulted in 2-5x 10^6 clones. The clones were scraped off the LB plates and frozen without further amplification.

Example 3: Identification of highly immunogenic peptide sequences from S. aureus using bacterial surface displayed genomic libraries and human serum

Experimental procedures

MACS screening. Approximately 2.5x 10^8 cells from a given library were grown in 5 ml LB-medium supplemented with 50 µg/ml Kanamycin for 2 h at 37°C. Expression was induced by the addition of 1 mM IPTG for 30 min. Cells were washed twice with fresh LB medium and approximately 2x 10^7 cells re-suspended in 100 µl LB medium and transferred to an Eppendorf tube.

10 µg of biotinylated, human serum was added to the cells and the suspension incubated over night at 4°C with gentle shaking. 900 µl of LB medium was added, the suspension mixed and subsequently centrifuged for 10 min at 6000 rpm at 4°C. Cells were washed once with 1 ml LB and then re-suspended in 100 µl LB medium. 10 µl of MACS microbeads coupled to streptavidin (Miltenyi Biotech, Germany) were added and the incubation continued for 20 min at 4°C. Thereafter 900 µl of LB medium was added and the MACS microbead cell suspension was loaded onto the equilibrated MS column (Mil-

tenyi Biotech, Germany) which was fixed to the magnet. (The MS columns were equilibrated by washing once with 1 ml 70% EtOH and twice with 2 ml LB medium.)

The column was then washed three times with 3 ml LB medium. The elution was performed by removing the magnet and washing with 2 ml LB medium. After washing the column with 3 ml LB medium, the 2 ml eluate was loaded a second time on the same column and the washing and elution process repeated. The loading, washing and elution process was performed a third time, resulting in a final eluate of 2 ml.

A second round of screening was performed as follows. The cells from the final eluate were collected by centrifugation and re-suspended in 1 ml LB medium supplemented with 50 µg/ml Kanamycin. The culture was incubated at 37°C for 90 min and then induced with 1 mM IPTG for 30 min. Cells were subsequently collected, washed once with 1 ml LB medium and suspended in 10 µl LB medium. Since the volume was reduced, 1 µg of human, biotinylated serum was added and the suspension incubated over night at 4°C with gentle shaking. All further steps were exactly the same as in the first selection round. Cells selected after two rounds of selection were plated onto LB-agar plates supplemented with 50 µg/ml Kanamycin and grown over night at 37°C.

Evaluation of selected clones by sequencing and Western blot analysis. Selected clones were grown over night at 37°C in 3 ml LB medium supplemented with 50 µg/ml Kanamycin to prepare plasmid DNA using standard procedures. Sequencing was performed at MWG (Germany) or in a collaboration with TIGR (U.S.A.).

For Western blot analysis approximately 10 to 20 µg of total cellular protein was separated by 10% SDS-PAGE and blotted onto HybondC membrane (Amersham Pharmacia Biotech, England). The LamB or FhuA fusion proteins were detected using human serum as the primary antibody at a dilution of 1:5000 and anti human IgG antibodies coupled to HRP at a dilution of 1:5000 as secondary antibodies. Detection was performed using the ECL detection kit (Amersham Pharmacia Biotech, England). Alternatively, rabbit anti FhuA or mouse anti LamB antibodies were used as primary antibodies in combination with the respective secondary antibodies cou-

pled to HRP for the detection of the fusion proteins.

Results

Screening of bacterial surface display libraries by magnetic activated cell sorting (MACS) using biotinylated human serum. The libraries LSA50/6 in pMAL9.1 and LSA250/1 in pHIE11 were screened with a pool of biotinylated, human patient sera (see Example 1) Preparation of antibodies from human serum). The selection procedure was performed as described under Experimental procedures. As a control, pooled human sera from infants that have most likely not been infected with S. aureus was used. Under the described conditions between 10 and 50 fold more cells with the patient compared to the infant serum were routinely selected (Figure 3). To evaluate the performance of the screen, approximately 100 selected clones were picked randomly and subjected to Western blot analysis with the same pooled patient serum. This analysis revealed that 30 to 50% of the selected clones showed reactivity with antibodies present in patient serum whereas the control strain expressing LamB or FhuA without a S. aureus specific insert did not react with the same serum. Colony PCR analysis showed that all selected clones contained an insert in the expected size range.

Subsequent sequencing of a larger number of randomly picked clones (500 to 800 per screen) led to the identification of the gene and the corresponding peptide or protein sequence that was specifically recognized by the human patient serum used for screening. The frequency with which a specific clone is selected reflects at least in part the abundance and/or affinity of the specific antibodies in the serum used for selection and recognizing the epitope presented by this clone. In that regard it is striking that some clones (ORF2264, ORF1951, ORF0222, lipase and IsaA) were picked up to 90 times, indicating their highly immunogenic property. All clones that are presented in Table 2 have been verified by Western blot analysis using whole cellular extracts from single clones to show the indicated reactivity with the pool of human serum used in the screen.

It is further worth noticing that most of the genes identified by the bacterial surface display screen encode proteins that are ei-

ther attached to the surface of S. aureus and/or are secreted. This is in accordance with the expected role of surface attached or secreted proteins in virulence of S. aureus.

Assessment of reactivity of highly immunogenic peptide sequences with different human sera. 10 to 30 different human patient sera were subsequently used to evaluate the presence of antibodies against the selected immunogenic peptide sequences that have been discovered in the screen according to the present invention. To eliminate possible cross-reactivity with proteins expressed by E. coli, all sera were pre-adsorbed with a total cellular lysate of E. coli DHa cells expressing FhuA protein.

This analysis is summarized in Table 2 and as an example shown in Figure 4 and is indicative of the validity of the present screen. It further shows that already short selected epitopes can give rise to the production of antibodies in a large number of patients (ORF1618, ORF1632, IsaA, Empbp, Protein A). Those peptide sequences that are not recognized by a larger set of patient sera may still be part of an highly immunogenic protein, but the recombinant protein itself may be tested for that purpose for each single case.

Example 4: Identification of highly immunogenic peptide sequences from genomic fragments from S. aureus using ribosome display and human serum

Experimental procedures

Ribosome display screening: 2.4 ng of the genomic library from S. aureus LSA250/1 in pMAL4.1 (described above) was PCR amplified with oligos ICC277 and ICC202 in order to be used for ribosome display. Oligos ICC277 (CGAATAATACGACTCACTATAGGGAGACCACAACGGTTTCCCACTAGTAATAATTTTGTTTAACTTTAAGAAGGAGATATATCCATGCAGaCCTTGGCCGGCCTCCC) and ICC202 (GGCCCACCCGTGAAGGTGAGCCGGCGTAAGATGCTTTTCTGTGACTGG) hybridize 5' and 3' of the Fse I-Not I insertion site of plasmid pMAL4.1, respectively. ICC277 introduces a T7 phage RNA polymerase promoter, a palindromic sequence resulting in a stem-loop structure on the RNA level, a ribosome binding site (RBS) and the translation start of gene 10 of the T7 phage including the ATG start codon.

Oligo ICC202 hybridizes at nucleotide position 668 of the ß-lactamase open reading frame and also introduces a stem-loop structure at the 3' end of the resulting RNA. PCR was performed with the High fidelity PCR kit (Roche Diagnostic) for 25 cycles at 50°C hybridization temperature and otherwise standard conditions.

The resulting PCR library was used in 5 consecutive rounds of selection and amplification by ribosome display similar as described previously (Hanes et al., 1997) but with modifications as described below.

One round of ribosome display contained the following steps: In vitro transcription of 2 µg PCR product with the RiboMax kit **(Promega)** resulted in ca. 50 µg A. In vitro translation was performed for 9 minutes at 37°C in 22 µl volume with 4.4 µl Premix Z (250 mM TRIS-acetate pH 7.5, 1.75 mM of each amino acid, 10 mM ATP, 2.5 mM GTP, 5 mM cAMP, 150 mM acetylphosphate, 2.5 mg/ml E. coli tRNA, 0.1 mg/ml folinic acid, 7.5 % PEG 8000, 200 mM potassium glutamate, 13.8 mM Mg(Ac)2, 8 µl S30 extract (x mg/ml) and about 2 µg in vitro transcribed RNA from the pool. S30 extract was prepared as described (Chen et al, 1983). Next, the sample was transferred to an ice-cold tube containing 35.2 µl 10 % milk-WBT (TRIS-acetate pH 7.5, 150 mM NaCl, 50 mM Mg(Ac)2, 0.1 % Tween-20, 10 % milk powder) and 52.8 µl WBTH (as before plus 2.5 mg/ml heparin). Subsequently, immuno precipitation was performed by addition of 10 µg purified IgGs, incubation for 90 minutes on ice, followed by addition of 30 µl MAGmol Protein G beads (Miltenyi Biotec, 90 minutes on ice). The sample was applied to a pre-equilibrated µ column (Miltenyi Biotec) and washed 5 times with ice-cold WBT buffer. Next 20 µl EB20 elution buffer (50 mM TRIS-acetate, 150 mM NaCl, 20 mM EDTA, 50 µg/ml S. cerevisiae RNA) was applied to the column, incubated for 5 minutes at 4°C. Elution was completed by adding 2 x 50 µl EB20. The mRNA from the elution sample was purified with the High pure RNA isolation kit (Roche Diagnostics). Subsequent reverse transcription was performed with Superscript II reverse transcriptase kit (Roche Diagnostics) according to the instruction of the manufacturer with 60 pmol oligo ICC202 for 1 hour at 50°C in 50 µl volume. 5 µl of this mix was used for the following PCR reaction with primers ICC202 and ICC277 as described above.

Three rounds of ribosome display were performed and the resulting selected PCR pool subsequently cloned into plasmid pHIE11 (described above) by cleavage with restriction endonucleases NotI and FseI.

Evaluation of selected clones by sequencing and peptide-ELISA analysis: Selected clones were grown over night at 37°C in 3 ml LB medium supplemented with 50 µg/ml Kanamycin to prepare plasmid DNA using standard procedures. Sequencing was performed at MWG (Germany) or at the Institute of Genomic Research (TIGR; Rockville, MD, U.S.A.). Peptides corresponding to the inserts were synthesized and coated in 10 mM $NaHCO_3$ pH 9.3 at a concentration of 10 µg/ml (50 µl) onto 96-well microtiter plates (Nunc). After blocking with 1% BSA in PBS at 37°C, 1:200 and 1:1000 dilutions of the indicated sera were diluted in 1% BSA/PBS and applied to the wells. After washing with PBS/0.1 % Tween-20, biotin-labeled anti-human IgG secondary antibodies (SBA) were added and these were detected by subsequent adding horseradish-peroxidase-coupled streptavidin according to standard procedures.

Results

The 250-bp genomic library (LSA250/1) as described above was used for screening. Purified IgGs from uninfected adults but with high titer against S. aureus as described above were used for selection of antigenic peptides.

Three rounds of ribosome display selection and amplificaton were performed according to Experimental procedures; finished by cloning and sequencing the resulting PCR pool.

Sequence analyses of a large number of randomly picked clones (700) led to the identification of the gene and the corresponding peptide or protein sequence that was specifically recognized by the high titer serum used for screening. The frequency with which a specific clone was selected reflects at least in part the abundance and/or affinity of the specific antibodies in the serum used for selection and recognizing the epitope presented by this clone. Remarkably, some clones (ORFs) were picked up to 50 times, indicating their highly immunogenic property. Table 2 shows the ORF name, the Seq.ID No. and the number of times it was identi-

fied by the inventive screen.

For a number of immuno-selected ORFs peptides corresponding to the identified immunogenic region were synthesized and tested in peptide-ELISA for their reactivity towards the sera pool they were identified with and also a number of additional sera from patients who suffered from an infection by S. aureus. The two examples in the graphs in figure 5 show the values of peptides from aureolysin and Pls. They are not only hyperimmune reactive against the high titer sera pool but also towards a number of individual patient's sera. All synthesized peptides corresponding to selected immunogenic regions showed reactivity towards the high titer sera pool and Table 2 summarizes the number of times the peptides were reactive towards individual patients sera, similar as described above.

In addition, it is striking that for those ORFs that were also identified by bacterial surface display described above), very often the actual immunogenic region within the ORF was identical or overlapping with the one identified by ribosome display. This comparison can be seen in Table 2.

Example 5: Identification of highly immunogenic antigens from S. aureus using Serological Proteome Analysis.

Experimental procedures

Surface protein preparations from S. aureus containing highly immunogenic antigens. S. aureus strains COL (Shafer and Iandolo, 1979) and agr- (Recsei et al., 1986) were stored as glycerol stocks at -80°C or on BHI (DIFCO) plates at 4°C. Single clones were used for inoculation of overnight cultures in either BHI ("standard conditions") or RPMI 1640 (GibcoBRL), last one depleted from iron ("stress conditions") by treating o/n with iminodiacetic acid (Sigma). Fresh medium was inoculated 1:100 the next day and bacteria were grown to $O.D._{600}$ between 0.3 and 0.7. Bacteria were harvested by centrifugation and washed with ice-cold PBS. Surface proteins were prepared by lysostaphin treatment under isotonic conditions (Lim et al. 1998). Briefly, ~3x 10^9 bacteria (according to $O.D._{600}$ = 1 are about 5x10^7 bacteria) were re-

suspended in 1 ml digestion buffer containing 35% raffinose (Aldrich Chemical Company), protease inhibitors (Roche) and 5 units lysostaphin (Sigma). After incubation at 37°C for 30 min, protoplasts were carefully sedimented by low-speed centrifugation. This treatment releases surface proteins covalently linked to the pentaglycine bridge of the peptidoglycan cell wall to the supernatant (in Crossley, 1997). Cell surface proteins were either precipitated with methanol/chlorophorm (Wessel, 1984) or concentrated in centrifugal filter-tubes (Millipore). Protein samples were frozen and stored at -80°C or dissolved in sample buffer and used for isoelectric focusing (IEF) immediately (Pasquali et al. 1997).

Serological proteome analysis of surface protein preparations from S. aureus. Samples were obtained from a) S. aureus/agr grown under "stress conditions", b) S. aureus/COL grown under "standard conditions" and c) S. aureus/COL "stress conditions". Loading onto 17 cm-strips containing immobilized pH gradients (pH 4-7, BioRad) was done using the "in-gel-reswelling procedure" (Pasquali et al., 1997). The gels for blotting were loaded with 100-800 µg protein, the preparative gels with 400-1,000 µg protein. Isoelectric focusing and SDS-PAGE (9-16% gradient gels) were performed as described (Pasquali et al., 1997). For Western blotting, proteins were transferred onto PVDF-membranes (BioRad) by semi-dry blotting. Transfer-efficiency was checked by amidoblack staining. After blocking (PBS/0.1% Tween 20/10% dry milk, 4°C for 16 h), blots were incubated for two hours with serum (1:2,500-1:100,000 in blocking solution, see Table 3). After washing, specific binding of serum IgG was visualized with a goat-anti-human-IgG / peroxidase conjugate (1:25.,000, Southern Biotech) as secondary antibody and development with a chemiluminescence substrate (ECL™, Amersham). A representative result is shown in Figure 6. Membranes were stripped by treatment with 2% ß-ME/Laemmli buffer for 30 min at 50-65°C, immediately re-probed with a different serum, and developed as described above. This procedure was repeated up to five times. Signals showing up with patient and/or healthy donor control sera but not with the infant pool, were matched to the Coomassie (BioRad) stained preparative gels (example shown in Figure 7). The results of these serological proteome analyses of surface protein preparations from S. aureus are summarized in Table 3.

Sequencing of protein spots by peptide-fingerprint MALDI-TOF-MS and tandem MS/MS. Gel pieces were washed alternately three times with 10 µl digestion buffer (10mM NH_4HCO_3/CAN, 1:1). Afterwards the gel pieces were shrunken with 10 µl ACN and reswollen with 2 µl protease solution (0.05 µg/µl trypsin, Promega, Madison, USA). Digestion was performed for 10-12 h at 37°C. For MALDI-TOF-MS peptides were extracted from the gel pieces with 10 µl digestion buffer. The supernatant was concentrated with ZipTip™ (Millipore, Bedford, USA), the peptides were eluted onto the MALDI target with 0.5 µl extraction buffer (0.1% TFA/CAN, 1:1) and 0.5 µl matrix solution (HCCA in ACN/0.1% TFA, 1:1) was added. MALDI-TOF-MS was done using a REFLEX III (Bruker Daltonik, Bremen, Germany) equipped with a SCOUT384 ion source. The acceleration voltage was set to 25 kV, and the reflection voltage to 28.7 kV. The mass range was set from 700 Da to 4000 Da. Data acquisition was done on a SUN Ultra using XACQ software, version 4.0. Post-analysis data processing was done using XMASS software, version 4.02 (Bruker Daltonik, Bremen, Germany). The results are summarized in tables 3 and 4.

Example 6: Characterisation of highly immunogenic proteins from S. aureus

The antigens identified by the different screening methods with the IgG and IgA preparations form pre-selected sera are further characterized, by the following ways:

1. The proteins are purified, most preferably as recombinant proteins expressed in E. coli or in a Gram+ expression system or in an in vitro translation system, and evaluated for antigenicity by a series of human sera. The proteins are modified based on bioinformatic analysis: N-terminal sequences representing the signal peptide are removed, C-terminal regions downstream of the cell wall anchor are also removed, and extra amino acids as tags are introduced for the ease of purification (such as Strep-tagII, His-tag, etc.) A large number of sera is then used in ELISA assays to assess the fraction of human sera containing specific antibodies against the given protein (see Fig. 9 as an example). One of the selected antigens is a 895 aa long protein, what was called LPXTGV (see Tables 2 and 4), since it contains the Gram+ cell wall anchor sequence LPXTG. This signature has been shown to

serve as cleavage site for sortase, a trans-peptidase which covalently links LPXTG motif containing proteins to the peptidoglycan cell wall. LPXTGV is also equipped with a typical signal peptide (Fig. 8). ELISA data using this protein as a Strep-tagged recombinant protein demonstrate that this protein is highly immunogenic (high titers relative to other recombinant proteins) in a high percentage of sera (Fig. 9). Importantly, patients with acute S. aureus infection produce significantly more of these anti-LPXTGV antibodies, than healthy normals, suggesting that the protein is expressed during in vivo infection. The overall ELISA titers of the individual antigenic proteins are compared, and the ones inducing the highest antibody levels (highly immunogenic) in most individuals (protein is expressed by most strains in vivo) are favored. Since the antigen specificity and quality (class, subtype, functional, nonfunctional) of the antibodies against S. aureus produced in individual patients can vary depending on the site of infection, accompanying chronic diseases (e.g. diabetes) and chronic conditions (e.g. intravascular device), and the individuals' immune response, special attention was paid to the differences detected among the different patient groups, since medical records belonging to each sera were available. In addition, each patient serum is accompanied by the pathogenic strain isolated from the patient at the time of serum sampling.

2. Specific antibodies are purified for functional characterization. The purity and the integrity of the recombinant proteins are checked (e.g. detecting the N-terminal Strep-tag in Western blot analysis in comparison to silver staining in SDS-PAGE). The antigens are immobilized through the tags to create an affinity matrix, and used for the purification of specific antibodies from highly reactive sera. Using as an example strep-tagged LPXTGV as the capture antigen, 20 µg of antibody from 125 mg of IgG were purified. Based on the ELISA data a pure preparation was received, not having e.g. anti-LTA and anti-peptidoglycan (both dominant with unfractionated IgG) activity. The antibodies are then used to test cell surface localization by FACS and fluorescent microscopy (Fig. 10).

3. Gene occurrence in clinical isolates

An ideal vaccine antigen would be an antigen that is present in all, or the vast majority of, strains of the target organism to

which the vaccine is directed. In order to establish whether the genes encoding the identified Staphylococcus aureus antigens occur ubiquitously in S. aureus strains, PCR was performed on a series of independent S. aureus isolates with primers specific for the gene of interest. S. aureus isolates were obtained from patients with various S. aureus infections. In addition several nasal isolates from healthy carriers and several lab strains were also collected and analyzed. The strains were typed according to restriction fragment length polymorphism (RFLP) of the spa and coa genes (Goh et al. 1992, Frénay et al., 1994, vanden Bergh et al. 1999). From these results 30 different strains were identified - 24 patient isolates, 3 nasal isolates and 3 lab strains. To establish the gene distribution of selected antigens, the genomic DNA of these 30 strains was subjected to PCR with gene specific primers that flank the selected epitope (ORF1361: Seq.ID No. 187 and 188; ORF2268: Seq.ID No. 193 and 194; ORF1951: Seq.ID No. 195 and 196; ORF1632: Seq.ID No. 181 and 182; ORF0766: Seq.ID No. 183 and 184; ORF0576: Seq.ID No. 185 and 186; ORF0222: Seq.ID No. 189 and 190; ORF0360: Seq.ID No. 191 and 192). The PCR products were analyzed by gel electrophoresis to identify a product of the correct predicted size. ORFs 1361, 2268, 1951, 1632, 0766 and 0222 are present in 100% of strains tested and ORF0576 in 97%. However ORF0360 occurred in only 71% of the strains. Thus ORFs 1361, 2268, 1951, 1632, 0766, 0576 and 0222 each have the required ubiquitous presence among S. aureus isolates.

These antigens (or antigenic fragments thereof, especially the fragments identified) are especially preferred for use in a vaccination project against S. aureus.

4. Identification of highly promiscuous HLA-class II helper epitopes within the ORFs of selected antigens

The ORFs corresponding to the antigens identified on the basis of recognition by antibodies in human sera, most likely also contain linear T-cell epitopes. Especially the surprising finding in the course of the invention that even healthy uninfected, non-colonized individuals show extremely high antibody titers (> 100,000 for some antigens, see Example 5) which are stable for >1 year (see Example 1), suggests the existence of T-cell dependent memory most probably mediated by CD4+ helper-T-cells. The molecular

definition of the corresponding HLA class II helper-epitopes is usefull for the design of synthetic anti-staphylococcal vaccines, which can induce immunological memory. In this scenario the helper-epitopes derived from the staphylococcal antigens provide "cognate help" to the B-cell response against these antigens or fragments thereof. Moreover it is possible to use these helper-epitopes to induce memory to T-independent antigens like for instance carbohydrates (conjugate vaccines). On the other hand, intracellular occurring staphylococci can be eliminated by CD8+ cytotoxic T-cells, which recognize HLA class I restricted epitopes.

T-cell epitopes can be predicted by various public domain algorithms: http://bimas.dcrt.nih.gov/molbio/hla_bind/ (Parker et al. 1994), http://134.2.96.221/scripts/MHCServer.dll/home.htm (Rammensee at al. 1999), http://mypage.ihost.com/usinet.hamme76/ (Sturniolo et al. 1999). The latter prediction algorithm offers the possibility to identify promiscuous helper-epitopes, i.e. peptides that bind to several HLA class II molecules. In order to identify highly promiscuous helper-epitopes within staphylococcal antigens the ORFs corresponding to Seq ID **64** (IsaA), Seq ID **114** (POV2), Seq ID **89** (ORF0222), Seq ID **70** (LPXTGIV), Seq ID **56** (LPXTGV), Seq ID **142** (LPXTGVI), Seq ID **81** (ORF3200), Seq ID **74** (ORF1951), Seq ID **94** (Empbp), Seq ID **83** (autolysin) and Seq ID **58** (ORF2498) were analyzed using the TEPITOPE package http://mypage.ihost.com/usinet.hamme76/ (Sturniolo et al. 1999). The analysis was done for 25 prevalent DR-alleles and peptides were selected if they were predicted to be a) strong binders (1% threshold) for at least 10/25 alleles or b) intermediate (3% threshold) binders for at least 17/25 alleles.
The following peptides containing one or several promiscuous helper-epitopes were selected (and are claimed):

Seq ID **56**:	pos. 6-40, 583-598, 620-646, 871-896
Seq ID **58**:	no peptide fulfills selection criteria
Seq ID **64**:	no peptide fulfills selection criteria
Seq ID **70**:	pos. 24-53
Seq ID **74**:	pos. 240-260
Seq ID **81**:	pos. 1660-1682, 1746-1790
Seq ID **83**:	pos. 1-29, 680-709, 878-902

Seq ID **89**: pos. 96-136
Seq ID **94**: pos. 1-29, 226-269, 275-326
Seq ID **114**: pos. 23-47, 107-156
Seq ID **142**: pos. 24-53

The corresponding peptides or fragments thereof (for instance overlapping 15-mers) can be synthesized and tested for their ability to bind to various HLA molecules in vitro. Their immunogenicity can be tested by assessing the peptide (antigen)-driven proliferation (BrdU or 3H-thymidine incorporation) or the secretion of cytokines (ELIspot, intracellular cytokine staining) of T-cells in vitro (Mayer et al. 1996, Schmittel et al. 2000, Sester et al. 2000). In this regard it will be interesting to determine quantitative and qualitative differences in the T-cell response to the staphylococcal antigens or the selected promiscuous peptides or fragments thereof in populations of patients with different staphylococcal infections, or colonization versus healthy individuals neither recently infected nor colonized. Moreover, a correlation between the antibody titers and the quantity and quality of the T-cell response observed in these populations is expected. Alternatively, immunogenicity of the predicted peptides can be tested in HLA-transgenic mice (Sonderstrup et al. 1999).

Similar approaches can be taken for the identification of HLA class I restricted epitopes within staphylococcal antigens.

Synthetic peptides representing one or more promiscuous T helper epitopes from S.aureus

Partially overlapping peptides spanning the indicated regions of Seq ID **56** (LPXTGV), Seq ID **70** (LPXTGIV), Seq ID **74** (ORF1hom1), Seq ID **81** (EM_BP), Seq ID **83** (Autolysin), Seq ID **89** (ORF1hom2), Seq ID **94** (EMPBP), Seq ID **114** (POV2) and Seq ID **142** (LPXTGVI) were synthesized. Sequences of the individual peptides are given in Table 5. All peptides were synthesized using Fmoc chemistry, HPLC purified and analyzed by mass spectrometry. Lyophilized peptides were dissolved in DMSO and stored at -20°C at a concentration of 5-10 mM.

Binding of synthetic peptides representing promiscuous T helper

epitopes to HLA molecules in vitro

Binding of peptides to HLA molecules on the surface of antigen-presenting cells is a prerequisite for activation of T cells. Binding was assessed in vitro by two independent biochemical assays using recombinant soluble versions of HLA class II molecules. One assay measures the concentration dependent competitive replacement of a labeled reference peptide by the test peptides. The second assay is based on the formation of SDS-stable complexes upon binding of high- and intermediate affinity ligands. A summary of the results obtained by the two assays is given in Table 5.

Soluble HLA molecules (DRA1*0101/DRB1*0101 and DRA1*0101/DRB1*0401) were expressed in SC-2 cells and purified as described in Aichinger et al., 1997. For the competition assay (Hammer et al. 1995) HLA molecules were applied between 50 and 200 ng/well. For DRB1*0101 biotinilated indicator peptide HA (PKYVKQNTLKLAT, Valli et al. 1993) was used at 0.008 µM. For DRB1*0401 biotinilated indicator peptide UD4 (YPKFVKQNTLKAA, Valli et al. 1993) was used between 0.03 and 0.06 µM. Test peptides were used in serial dilutions from 0.02 nM to 200 µM. Molecules, indicator and test peptides were incubated overnight at 37°C, pH 7. HLA:peptide complexes obtained after incubation with serial dilutions of test and reference peptides (the known high-affinity binders HA and UD4 were used as positive control) were captured in ELISA plates coated with antibody L243, which is known to recognize a conformational epitope formed only by correctly associated heterodimers. Incorporated biotin was measured by standard colorimetric detection using a streptavidin-alkaline phosphatase conjugate (Dako) with NBT/BCIP tablets (Sigma) as substrate and automated OD reading on a Victor reader (Wallac).

T cell response against promiscuous T helper epitopes assessed by IFNg ELIspot assay

Upon antigenic stimulation T cells start to proliferate and to secrete cytokines such as interferon gamma (IFNg). Human T cells specifically recognizing epitopes within S.aureus antigens were detected by IFNg-ELIspot (Schmittel et al. 2000). PBMCs from healthy individuals with a strong anti-S.aureus IgG response were isolated from 50-100 ml of venous blood by ficoll density gradi-

ent centrifugation and used after freezing and thawing. Cells were seeded at 200,000/well in 96-well plates. Peptides were added as mixtures corresponding to individual antigens, in both cases at 10 µg/ml each. Concanavalin A (Amersham) and PPD (tuberculin purified protein derivate, Statens Serum Institute) served as assay positive controls, assay medium without any peptide as negative control. After overnight incubation in Multi Screen 96-well filtration plates (Millipore) coated with the anti-human IFNg monoclonal antibody B140 (Bender Med Systems) the ELIspot was developed using the biotinylated anti-human IFNg monoclonal antibody B308-BT2 (Bender Med Systems), Streptavidin-alkaline phosphatase (DAKO) and BCIP/NBT alkaline phosphatase substrate (SIGMA). Spots were counted using an automatic plate reader (Bioreader 2000, BIO-SYS). Spots counted in wells with cells stimulated with assay medium only (negative control, generally below 10 spots / 100.000 cells) were regarded as background and subtracted from spot numbers counted in wells with peptides.

Table 5: Promiscuous T helper epitopes contained in S.aureus antigens

Amino acid sequences within S.aureus antigens containing highly promiscuous T helper epitopes	binding [1]	IFNg ELIspot [2]
Seq ID **56** (LPXTGV): pos. 6-40 p6-28 >PKLRSFYSIRKSTLGVASVIVST// p24-40 >VIVSTLFLISQHQAQA//	+ –	44;80;8 ;95;112
Seq ID **56** (LPXTGV): pos. 620-646 p620-646 >FPYIPDKAVYNAIVKVVVANIGYEGQ//	+	
Seq ID **56** (LPXTGV): pos. 871-896 p871-896 >QSWWGLYALLGMLALFIPKFRKESK//	–	
Seq ID **70** (LPXTGIV): pos. 24-53 p24-53 >YSIRKFTVGTASILIGSLMYLGTQQEAEA//	nd	34;14;0 ;57;16
Seq ID **74** (ORF1hom1): pos. 240-260 p240-260 >MNYGYGPGVVTSRTISASQA//	+	47;50;0 ;85;92

Seq ID **81** (EM_BP): pos. 1660-1682 p1660-1682 >NEIVLETIRDINNAHTLQQVEA//	nd	2;14;5;77;26
Seq ID **81** (EM_BP): pos. 1746-1790 p1746-1773 >LHMRHFSNNFGNVIKNAIGVVGISGLLA// p1753-1779 >NNFGNVIKNAIGVVGISGLLASFWFFI// p1777-1789 >FFIAKRRRKEDEE/	nd nd nd	
Seq ID **83** (Autolysin) pos. 1-29 p1-29: >MAKKFNYKLPSMVALTLVGSAVTAHQVQA//	nd	6;35;7;60;49
Seq ID **83** (Autolysin) pos. 878-902 p878-902: >NGLSMVPWGTKNQVILTGNNIAQG/	nd	
Seq ID **89** (ORF1hom2): pos. 96-136 p96-121 >GESLNIIASRYGVSVDQLMAANNLRG// p117-136 >NNLRGYLIMPNQTLQIPNG//	- -	0;35;0;29;104
Seq ID **94** (EMPBP): pos. 1-29 p4-29 : >KLLVLTMSTLFATQIMNSNHAKASV//	+	
Seq ID **94** (EMPBP): pos. 226-269 p226-251 >IKINHFCVVPQINSFKVIPPYGHNS// p254-270 >MHVPSFQNNTTATHQN//	- +	26;28;16;43;97
Seq ID **94** (EMPBP): pos. 275-326 p275-299 >YDYKYFYSYKVVKGVKKYFSFSQS// p284-305 >YKVVKGVKKYFSFSQSNGYKIG// p306-326 >PSLNIKNVNYQYAVPSYSPT//	+ + +	
Seq ID **114** (POV2): pos. 23-47 p23-47 >AGGIFYNQTNQQLLVLCDGMGGHK//	-	49;20;4;77;25
Seq ID **114** (POV2): pos. 107-156 p107-124 >ALVFEKSVVIANVGDSRA/ p126-146 >RAYVINSRQIEQITSDHSFVN// p142-158 >SFVNHLVLTGQITPEE//	- nd nd	
Seq ID **142** (LPXTGVI): pos. 1-42 p6-30 >KEFKSFYSIRKSSLGVASVAISTL// p18-42 >SSLGVASVAISTLLLLMSNGEAQA//	++ nd	0;41;20;88;109
Seq ID **142** (LPXTGVI): pos. 209-244 p209-233 >IKLVSYDTVKDYAYIRFSVSNGTKA// p218-244 >KDYAYIRFSVSNGTKAVKIVSSTHFNN//	+ +	
Seq ID **142** (LPXTGVI): pos. 395-428 p395-418 >FMVEGQRVRTISTYAINNTRCTIF// p416-428 >TIFRYVEGKSLYE//	- -	

Seq ID **142** (LPXTGVI): pos. 623-647 p623-647 >MTLPLMALLALSSIVAFVLPRKRKN //	-	

[1] binding to soluble DRA1*0101/DRB1*0401 molecules was determined using a competition assay (+, ++: binding, -: no competition up to 200 µM test peptide; nd: not done)

[2] results from 5 healthy individuals with strong anti-S.aureus IgG response. Data are represented as spots/200.000 cells (background values are subtracted

5. Antigens may be injected into mice - and the antibodies against these proteins can be measured.

6. Protective capacity of the antibodies induced by the antigens through vaccination can be assessed in animal models.

Both 5. and 6. are methods well available to the skilled man in the art.

Example 7: Applications

A) An effective vaccine offers great potential for patients facing elective surgery in general, and those receiving endovascular devices, in particular. Patients suffering from chronic diseases with decreased immune responses or undergoing continuous ambulatory peritoneal dialysis are likely to benefit from a vaccine with S. aureus by immunogenic serum-reactive antigens according to the present invention. Identification of the relevant antigens will help to generate effective passive immunization (humanized monoclonal antibody therapy), which can replace human immunoglobulin administration with all its dangerous side-effects. Therefore an effective vaccine offers great potential for patients facing elective surgery in general, and those receiving endovascular devices, in particular.

S. aureus can cause many different diseases.

1. Sepsis, bacteriaemia
2. Haemodialysed patients - bacteriemia, sepsis
3. Peritoneal dialyses patients - peritonitis
4. Patients with endovascular devices (heart surgery,etc) - endocarditis, bacteriemia, sepsis

5. Orthopedic patients with prosthetic devices - septic arthritis
6. Preventive vaccination of general population

B) Passive and active vaccination, both with special attention to T-cells with the latter one: It is an aim to induce a strong T helper response during vaccination to achieve efficient humoral response and also immunological memory. Up till now, there is no direct evidence that T-cells play an important role in clearing S. aureus infections, however, it was not adequately addressed, so far. An effective humoral response against proteinaceous antigens must involve T help, and is essential for developing memory. Naïve CD4+ cells can differentiated into Th1 or Th2 cells. Since, innate immunological responses (cytokines) will influence this decision, the involvement of T-cells might be different during an acute, serious infection relative to immunization of healthy individuals with subunit vaccines, not containing components which impair the immune response during the natural course of the infection. The consequences of inducing Th1 or Th2 responses are profound. Th1 cells lead to cell-mediated immunity, whereas Th2 cells provide humoral immunity.

C) Preventive and therapeutic vaccines
Preventive: active vaccination/passive immunization of people in high risk groups, before infection
Therapeutic: passive vaccination of the already sick.

Active vaccination to remove nasal carriage

Specific example for an application

Elimination of MRSA carriage and prevention of colonization of the medical staff

Carriage rates of S. aureus in the nares of people outside of the hospitals varies from 10 to 40%. Hospital patients and personnel have higher carriage rates. The rates are especially high in patients undergoing hemodialysis and in diabetics, drug addicts and patients with a variety of dermatologic conditions. Patients at highest risk for MRSA infection are those in large tertiary-care hospitals, particularly the elderly and immunocompromised, those

in intensive care units, burn patients, those with surgical wounds, and patients with intravenous catheters.

The ELISA data strongly suggest that there is a pronounced IgA response to S. aureus, which is not obvious or known from the literature. Since the predominant mucosal immune response is the production of IgA with neutralizing activity, it is clear that the staphylococcal epitopes and antigens identified with the highly pure IgA preparations lead to an efficient mucosal vaccine.

- Clear indication: Everybody's threat in the departments where they perform operation (esp. orthopedics, traumatology, gen. surgery)
- Well-defined population for vaccination (doctors and nurses)
- Health care workers identified as intranasal carriers of an epidemic strain of S. aureus are currently treated with mupirocin and rifampicin until they eliminate the bacteria. Sometimes it is not effective, and takes time.
- Available animal model: There are mice models for intranasal carriage.

Table 1: ELISA titers of sera from non-infected individuals against multiple staphylococcal proteins.

Sera ID#	BHI lysate	LTA	PG	ClfA	D1+D3	FnBPA	sdrE	sdrC	EBP	enolase	LP309	LP342	coagul	Fib	SrtA	ClfB	Map-w
1																	
2	2.....	2.....		8.....	4.....							6.....		3.....			
3	7.....			3.....			1.....	1.....			3.....	2.....	2.....	2.....	3.....	7.....	4.....
4	1******	1******	1.....	6.....	2.....	2.....	3.....	6.....	2.....	6,7.....	8.....	3.....			4.....	1.....	3.....
5																	
6								4.....			5.....						
7		6.....					7.....		7.....	5.....				4.....			7.....
8							8.....		8.....					5.....	7.....	8,9.....	
9	4,5,6....	4.....	5.....		5.....		6.....	3.....	1.....	3,4.....				1.....		5,6.....	
10																5,6.....	
11					6.....										6.....		
12													4,5.....				
13							5.....	2.....						8.....			
14																	
15	3.....	5.....	2,3.....			5.....					6.....	7.....			8.....		8,9.....
16																	6.....
17																	
18			6,7.....	1.....					3.....							4.....	
19																	1.....
20																	
21				2.....													2.....

Sera ID#	BHI lysate	LTA	PG	ClfA	D1+D3	FnBPA	sdrE	sdrC	EBP	enolase	LP309	LP342	coagul	Fib	SrtA	ClfB	Map-w
22																	
23	4,5,6....			5.....	3.....	6.....	2.....	7.....	4.....	6,7.....	7.....		6,7.....		2.....	2.....	
24							4.....		6.....								8,9.....
25			5.....														
26	8.....													7.....			
27								8.....			4.....	4,5.....	4,5.....		5.....		
28																	
29										1.....							
30																	
31					1.....	1.....							1.....				
32			4.....														
33			8.....	4.....		4.....		5.....									
34					7,8.....					2.....	2.....	1.....	6,7.....	6.....	1.....		
35	4,5,6....	8.....	2,3.....						5.....		1******					3.....	4.....
36		3.....															
37				7.....	7,8.....								3.....				
38				8.....						3,4.....							
39																	
40		7.....	6,7.....			3.....						4,5.....				8,9.....	

Table I. ELISA titers of sera from non-infected individuals against multiple staphylococcal proteins.

Anti-staphylococcal antibody levels were measured individually by standard ELISA with total lysate prepared from S. aureus grown in BHI medium (BHI), lipoteichoic acid (LTA), peptidoglycan (PG), 13 recombinant proteins, representing cell surface and secreted proteins, such as clumping factor A and B (ClfA, ClfB), Fibronectin-binding protein (FnBPA), SD-repeat proteins (sdrC, sdrE), MHC Class II analogous protein (map-w), Elastin-binding protein (EBP), enolase (reported to be cell surface located and immunogenic), iron transport lipoproteins (LP309, LP342), sortase (srtA), coagulase (coa), extracellular fibrinogen-binding protein (fib). Two short synthetic peptides representing 2 of the five immunodominant D repeat domains from FnBPA was also included (D1+D3) as antigens. The individual sera were ranked based on the IgG titer, and obtained a score from 1-9. Score 1 labels the highest titer serum and score 8 or 9 labels the sera which were 8th or 9th among all the sera tested for the given antigen. It resulted in the analyses of the top 20 percentile of sera (8-9/40). The five "best sera" meaning the most hyper reactive in terms of anti-staphylococcal antibodies were selected based on the number of scores 1-8. **** means that the antibody reactivity against the particular antigen was exceptionally high (>2x ELISA units relative to the 2nd most reactive serum).

Table 2a: Immunogenic proteins identified by bacterial surface and ribosome display: S. aureus

Bacterial surface display: A, LSA250/1 library in fhuA with patient sera 1 (655); B, LSA50/6 library in lamB with patient sera 1 (484); C, LSA250/1 library in fhuA with IC sera 1 (571); E, LSA50/6 library in lamB with IC sera 2 (454); F, LSA50/6 library in lamB with patient sera P1 (1105); G, LSA50/6 library in lamb with IC sera 1 (471)); H, LSA250/1 library in fhuA with patient sera 1 (IgA, 708). Ribosome display: D, LSA250/1 library with IC sera (1686). *, identified 18 times of 33 screened; was therefore eliminated from screen C. **, prediction of antigenic sequences longer than 5 amino acids was performed with the programme ANTIGENIC (Kolaskar and Tongaonkar, 1990); #, identical sequence present twice in ORF; ##, clone not in database (not sequence by

TIGR).

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
SaA0003	ORF2963P	repC	5–20, 37–44, 52–59, 87–94, 116–132	C:3	aa 112–189	C:GSBYM94(112–189):26/30	171, 172
SaA0003	ORF2967P	repC	7–19, 46–57, 85–91, 110–117, 125–133, 140–149, 156–163, 198–204, 236–251, 269–275, 283–290, 318–323, 347–363	C:18	aa 9–42 aa 158–174	C:GSBYI53(9–42):1/1	150, 158
0093	ORF1879	SdrC	23–51, 75–80, 90–99, 101–107, 151–157, 173–180, 186–205, 215–226, 239–263, 269–274, 284–304, 317–323, 329–336, 340–347, 360–366, 372–379, 391–397, 399–406, 413–425, 430–436, 444–455, 499–505, 520–529, 553–568, 586–592, 600–617, 631–639, 664–678, 695–701, 891–903, 906–912, 926–940	A:1, D:5, C:1, F:6, G:2	aa 98–182 aa 684–764 aa 836–870	A:GSBXL70(98–182):9/30 D:n.d. C:GSBYH73(815–870):3/16	34, 86
0095	ORF1881	SdrE	25–45, 72–77, 147–155, 198–211, 217–223, 232–238, 246–261, 266–278, 281–294, 299–304, 332–340, 353–360, 367–380, 384–396, 404–409, 418–429, 434–440, 448–460, 465–476, 493–509, 517–523, 531–540, 543–555, 561–566, 576–582, 584–591, 603–617, 633–643, 647–652, 668–674, 677–683, 696–704, 716–728, 744–752, 755–761, 789–796, 809–815, 826–840, 854–862, 887–903, 918–924, 1110–1116, 1125–1131, 1145–1159	C:12, E:2	aa 147–192	C:GSBYH31(147–192):2/14 E:GSBZA27(144–162):23/41	145, 153
0123	ORF1909	unknown	9–28, 43–48, 56–75, 109–126, 128–141, 143–162, 164–195, 197–216, 234–242, 244–251	B:3, E:7, G:1	aa 168–181	B:GSBXF80(168–181):5/27 E:GSBZC17(168–181):25/41	35, 87
0160	ORF1941	unknown	4–10, 20–42, 50–86, 88–98, 102–171, 176–182, 189–221, 223–244, 246–268, 276–284, 296–329	A:1	aa 112–188	A:GSBXO07(112–188):5/30	36, 88
0222	ORF1988	homology with ORF1	4–9, 13–24, 26–34, 37–43, 45–51, 59–73, 90–96, 99–113, 160–173, 178–184, 218–228, 233–238, 255–262	A:52, C:18*, H:19	aa 45–105 aa 103–166 aa 66–153	A:GSBXM63(65–95):1/1 A:GSBXM82(103–166):14/29 A:GSBXK44–bmd3(65–153):47/51	37, 89
0308	ORF2077	Complement, unknown	13–27, 42–63, 107–191, 198–215, 218–225, 233–250	A:6, B:2, C:47, E:35	complement bp 474–367	A:GSBXK03(bp473–367):28/69 B:GSBXD29(bp465–431):10/27	38, 90

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
0317	ORF2088	preprotein translocase secA subunit	16–29, 64–77, 87–93, 95–101, 127–143, 150–161, 204–221, 225–230, 236–249, 263–269, 281–309, 311–325, 337–343, 411–418, 421–432, 435–448, 461–467, 474–480, 483–489, 508–516, 542–550, 580–589, 602–611, 630–636, 658–672, 688–705, 717–723, 738–746, 775–786, 800–805, 812–821, 828–834	A:1	aa 1–19	A:GSBXP37(1–19):6/29	39, 91
0337	ORF2110	Hypothetical protein	26–53, 95–123, 164–176, 189–199	D:12	aa 8–48	D:n.d.	40, 92
0358	ORF2132	Clumping factor A	8–35, 41–48, 59–66, 87–93, 139–144, 156–163, 198–209, 215–229, 236–244, 246–273, 276–283, 285–326, 328–342, 349–355, 362–370, 372–384, 396–402, 405–415, 423–428, 432–452, 458–465, 471–477, 484–494, 502–515, 540–547, 554–559, 869–875, 893–898, 907–924	C:1, D:2, E:1	aa 706–809	D:n.d.	41, 93
0360	ORF2135 Empbp	extracellular matrix and plasma binding protein	7–13, 15–23, 26–33, 68–81, 84–90, 106–117, 129–137, 140–159, 165–172, 177–230, 234–240, 258–278, 295–319	A:46, B:21, C:11, E:2, F:18, G:7, H: 12	aa 22–56 aa 23–99 aa 97–115 aa 233–250 aa 245–265	A:GSBXK24(23–55):1/1 B:GSBXB43(39–54):58/71 A:GSBXK02–bmd1(22–99):59/59 B:GSBXD82–bdb19(97–115):1/1 F:SALAL03(233–250):15/41	42, 94
0453	ORF2227	coma operon protein 2	17–25, 27–55, 84–90, 95–101, 115–121	C:3	aa 55–101	C:GSBYG07(55–101):1/1	146, 154
0569	ORF1640	V8 protease	5–32, 66–72, 87–98, 104–112, 116–124, 128–137, 162–168, 174–183, 248–254, 261–266, 289–303, 312–331	A:1, F:1	aa 174–249	A:GSBXS51(174–249):11/30	32, 84

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
0576	ORF1633 Autolysin	autolysin, adhesion	4-19, 57-70, 79-88, 126-132, 144-159, 161-167, 180-198, 200-212, 233-240, 248-255, 276-286, 298-304, 309-323, 332-346, 357-366, 374-391, 394-406, 450-456, 466-473, 479-487, 498-505, 507-519, 521-530, 532-540, 555-565, 571-581, 600-611, 619-625, 634-642, 650-656, 658-665, 676-682, 690-699, 724-733, 740-771, 774-784, 791-797, 808-815, 821-828, 832-838, 876-881, 893-906, 922-929, 938-943, 948-953, 969-976, 1002-1008, 1015-1035, 1056-1069, 1105-1116, 1124-1135, 1144-1151, 1173-1181, 1186-1191, 1206-1215, 1225-1230, 1235-1242	A:21, B:46, C:55, E:5, F:85, H:19	aa 6-66 aa 65-124 aa 579-592 aa 590-604	A:GSBXN93(6-66):5/16 C:GSBYH05(45-144):7/8 A:GSBXK66-bmd18(65-124):16/30 B:GSBXB89(108-123):1/1 B:GSBXB02(590-603):39/71 F:SALAM15(579-592):25/41	31, 83
0657	ORF unknown	LPXTGVI protein	9-33, 56-62, 75-84, 99-105, 122-127, 163-180, 186-192, 206-228, 233-240, 254-262, 275-283, 289-296, 322-330, 348-355, 416-424, 426-438, 441-452, 484-491, 541-549, 563-569, 578-584, 624-641	A:2, B:27, F:15	aa 527-544	B:GSBXE07-bdb1(527-542):11/71 F:SALAX70(526-544):11/41	1, 142
0749	ORF1462	Carbamoyl-phosphate synthase	8-23, 31-38, 42-49, 61-77, 83-90, 99-108, 110-119, 140-147, 149-155, 159-171, 180-185, 189-209, 228-234, 245-262, 264-275, 280-302, 304-330, 343-360, 391-409, 432-437, 454-463, 467-474, 478-485, 515-528, 532-539, 553-567, 569-581, 586-592, 605-612, 627-635, 639-656, 671-682, 700-714, 731-747, 754-770, 775-791, 797-834, 838-848, 872-891, 927-933, 935-942, 948-968, 976-986, 1000-1007, 1029-1037	C:2	aa 630-700	C:GSBYK17(630-700):5/9	144, 152
944	ORF1414	Yfix	6-33, 40-46, 51-59, 61-77, 84-104, 112-118, 124-187, 194-248, 252-296, 308-325, 327-361, 367-393, 396-437, 452-479, 484-520, 535-545, 558-574, 582-614, 627-633, 656-663, 671-678, 698-704, 713-722, 725-742, 744-755, 770-784, 786-800, 816-822, 827-837	D:4	aa 483-511	D :n.d.	30, 82
1050	ORF1307	unknown	49-72, 76-83, 95-105, 135-146, 148-164, 183-205	A:1, H:45	aa 57-128	A:GSBXM26(57-128):7/30	28, 80

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
1209	ORF3006	hemN homolog	12–36, 43–50, 58–65, 73–78, 80–87, 108–139, 147–153, 159–172, 190–203, 211–216, 224–232, 234–246, 256–261, 273–279, 286–293, 299–306, 340–346, 354–366	B:7, F:8	aa 167–181	B:GSBXB76(167–179):25/71 F:SALBC54(169–183):18/41	54, 106
1344	ORF0212	NifS protein homolog	8–16, 22–35, 49–58, 70–77, 101–121, 123–132, 147–161, 163–192, 203–209, 216–234, 238–249, 268–274, 280–293, 298–318, 328–333, 339–345, 355–361, 372–381	A:11	aa 34–94	A:GSBXK59–bmd21(34–94):6/29	5, 141
1356	ORF0197	Hypothetical protease	28–55, 82–100, 105–111, 125–131, 137–143	D:12	aa 1–49	D:n.d.	4, 57
1361	ORF0190	LPXTGV protein	5–39, 111–117, 125–132, 134–141, 167–191, 196–202, 214–232, 236–241, 244–249, 292–297, 319–328, 336–341, 365–380, 385–391, 407–416, 420–429, 435–441, 452–461, 477–488, 491–498, 518–532, 545–556, 569–576, 581–587, 595–602, 604–609, 617–640, 643–651, 702–715, 723–731, 786–793, 805–811, 826–839, 874–889	A:1, B:23, E:3, F:31	aa 37–49 aa 63–77 aa 274–334	B:GSBXF81(37–49):1/1 B:GSBXD45–bdb4(62–77):12/70 A:GSBXL77(274–334):5/30 F:SALAP81(62–77):10/41	3, 56
1371	ORF0175	YtpT, conserved hypothetical protein	37–42, 57–62, 121–135, 139–145, 183–190, 204–212, 220–227, 242–248, 278–288, 295–30, 304–309, 335–341, 396–404, 412–433, 443–449, 497–503, 505–513, 539–545, 552–558, 601–617, 629–649, 702–711, 736–745, 793–804, 814–829, 843–858, 864–885, 889–895, 905–913, 919–929, 937–943, 957–965, 970–986, 990–1030, 1038–1049, 1063–1072, 1080–1091, 1093–1116, 1126–1136, 1145–1157, 1163–1171, 1177–1183, 1189–1196, 1211–1218, 1225–1235, 1242–1256, 1261–1269	C:3, E:2, G:1	aa 624–684 aa 891–905	C:GSBYG95(624–684):7/22 E:GSBZB45(891–905):10/41	143, 151
1491	ORF0053	Cmp binding factor 1 homolog	12–29, 34–40, 63–71, 101–110, 114–122, 130–138, 140–195, 197–209, 215–229, 239–253, 255–274	A:7, C:2, E:7, F:4	aa 39–94	A:GSBXM13(39–94):10/29 F:SALAY30(39–53):4/41	2, 55
1616	ORF1180	leukocidin F homolog	16–24, 32–39, 43–49, 64–71, 93–99, 126–141, 144–156, 210–218, 226–233, 265–273, 276–284	A:10	aa 158–220	A:GSBXK06(158–220):8/29	27, 79
1618	ORF1178	LukM homolog	5–24, 88–94, 102–113, 132–143, 163–173, 216–224, 254–269, 273–278, 305–313, 321–327, 334–341	A:13, B:3 C:36, E:4, F:12, G:2, H:10	aa 31–61 aa 58–74	A:GSBXK60(31–61):20/29 B:GSBXB48(58–74):49/71 F:SALAY41(58–74):30/41	26, 78

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
1632	ORF1163	SdrH homolog	7-35, 54-59, 247-261, 263-272, 302-320, 330-339, 368-374, 382-411	B:6, E:11, F:34	aa 105-119 aa 126-143 aa 168-186	B:GSBXG53(168-186):39/71 F:SALAP07(105-119):11/41	25, 77
1763	ORF1024	unknown	5-32, 35-48, 55-76	C:3	complement bp 237-170	C:GSBYI30(98aa):1/1	24, 76
1845	ORF0942	Hyaluronate lyase	10-26, 31-44, 60-66, 99-104, 146-153, 163-169, 197-205, 216-223, 226-238, 241-258, 271-280, 295-315, 346-351, 371-385, 396-407, 440-446, 452-457, 460-466, 492-510, 537-543, 546-551, 565-582, 590-595, 635-650, 672-678, 686-701, 705-712, 714-721, 725-731, 762-768, 800-805	D:5, F:2	aa208-224 aa 672-727	D:n.d.	23, 75
1951	ORF0831	homology with ORF1	5-22, 42-50, 74-81, 139-145, 167-178, 220-230, 246-253, 255-264	A:223, B:56, C:167, E:43, F:100, G:13, H:102	aa 137-237 aa 250-267	B:GSBXC07(180-190):1/1 A:GSBXK29(177-195):15/29 B:GSBXD43(250-267):10/71 F:SALAM13(178-191):20/41	22, 74
1955	ORF0826	homology with ORF1	4-9, 15-26, 65-76, 108-115, 119-128, 144-153	A:1, B:3, E:1, F:8	aa 38-52 aa 66-114	A:GSBXR10(66-114):5/30 F:SALAM67(37-52):16/41	21, 73
2031	ORF0749	unknown	10-26, 31-43, 46-58, 61-66, 69-79, 85-92, 100-115, 120-126, 128-135, 149-155, 167-173, 178-187, 189-196, 202-222, 225-231, 233-240, 245-251, 257-263, 271-292, 314-322, 325-334, 339-345	B:2, F:2	aa 59-74	B:GSBXC01(59-71):11/26	20, 72
2086	ORF0691 Sbi	IgG binding protein	6-20, 53-63, 83-90, 135-146, 195-208, 244-259, 263-314, 319-327, 337-349, 353-362, 365-374, 380-390, 397-405, 407-415	A:1, B:8, E:24, F:9, G:137	aa 208-287 aa 261-276 aa 286-314	A:GSBXS55(208-287):38/46 B:GSBXB34(299-314)::11/71 F:SALAX32(261-276):21/41	19, 71

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
2180	ORF0594	LPXTGIV protein	11-20, 26-47, 69-75, 84-92, 102-109, 119-136, 139-147, 160-170, 178-185, 190-196, 208-215, 225-233, 245-250, 265-272, 277-284, 300-306, 346-357, 373-379, 384-390, 429-435, 471-481, 502-507, 536-561, 663-688, 791-816, 905-910, 919-933, 977-985, 1001-1010, 1052-1057, 1070-1077, 1082-1087, 1094-1112	A:3, C:3, E:6, F:2, H: 6	aa 493-587 aa 633-715 aa 704-760[#] aa 760-832 (aa 832-887)[#]	A:GSBXS61(493-555):1/1 A:GSBXL64(496-585):1/1 A:GSBXS92(760-841):1/1 A:bmd4(704-760):16/30[#] (A:bmd4(830-885):16/30)[#] F:SALBC43(519-533):4/41	18, 70
2184	ORF0590	FnbpB	5-12, 18-37, 104-124, 139-145, 154-166, 175-181, 185-190, 193-199, 203-209, 235-244, 268-274, 278-292, 299-307, 309-320, 356-364, 375-384, 390-404, 430-440, 450-461, 488-495, 505-511, 527-535, 551-556, 567-573, 587-593, 599-609, 624-631, 651-656, 665-671, 714-726, 754-766, 799-804, 818-825, 827-833, 841-847, 855-861, 876-893, 895-903, 927-940	A:2, C:4, G:9	aa 701-777 aa 783-822	A:GSBXM62(702-777):28/28 A:GSBXR22(783-855):1/1	17, 69
2186	ORF0588	Fnbp	8-29, 96-105, 114-121, 123-129, 141-147, 151-165, 171-183, 198-206, 222-232, 253-265, 267-277, 294-300, 302-312, 332-338, 362-368, 377-383, 396-402, 410-416, 451-459, 473-489, 497-503, 537-543, 549-559, 581-600, 623-629, 643-649, 655-666, 680-687, 694-700, 707-712, 721-727, 770-782, 810-822, 874-881, 883-889, 897-903, 911-917, 925-931, 933-939, 946-963, 965-973, 997-1010	A:4, C:4, D:5, E:2	aa 710-787 aa 855-975 aa 916-983	C:GSBYN05(710-787):19/25 D:n.d. A:GSBXP01(916-983):17/30	16, 68
2224	ORF0551	unknown	49-56, 62-68, 83-89, 92-98, 109-115, 124-131, 142-159, 161-167, 169-175, 177-188, 196-224, 230-243, 246-252	B:2	aa 34-46	B:GSBXD89(34-46):1/1	15, 67

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
2254	ORF0519	Conserved hypothetical protein	14–22, 32–40, 52–58, 61–77, 81–93, 111–117, 124–138, 151–190, 193–214, 224–244, 253–277, 287–295, 307–324, 326–332, 348–355, 357–362, 384–394, 397–434, 437–460, 489–496, 503–510, 516–522, 528–539, 541–547, 552–558, 563–573, 589–595, 602–624, 626–632, 651–667, 673–689, 694–706, 712–739, 756–790	D:3	aa 403–462	D.n.d.	14, 66
2264	ORF0509	ORF1; homology with putative secreted antigen precursor from *S. epidermidis*	5–31, 47–55, 99–104, 133–139, 156–172, 214–224, 240–247	A:131, B:51, C:13, E:43, F:78, G:2, H:17	aa 7–87 aa 133–242	A:GSBXP22(145–196):1/1 A:GSBXK05–bmd16(178–218):6/29 B:GSBXE24–bdb20(167–178):1/1 F:SALAQ91(173–184):15/41	13, 65
2268	ORF0503	IsaA, possibly adhesion/ aggregation	7–19, 26–45, 60–68, 94–100, 111–119, 126–137, 143–148, 169–181, 217–228	A:7, B:65, C:3, E:2, F:53	aa 67–116 aa 98–184 aa 182–225	A:GSBXK88(67–116):1/1 A:GSBXN19(98–184):22/29 A:GSBXN32(182–225):34/71 B:GSBXB71(196–209):16/29 F:SALAL22(196–210):16/41	12, 64
2344	ORF0426	Clumping factor B	4–10, 17–45, 120–127, 135–141, 168–180, 187–208, 216–224, 244–254, 256–264, 290–312, 322–330, 356–366, 374–384, 391–414, 421–428, 430–437, 442–449, 455–461, 464–479, 483–492, 501–512, 548–555, 862–868, 871–876, 891–904	D:9, E:1, F:3, H: 4	aa 706–762 aa 810–852	D:n.d.	11, 63
2351	ORF0418	aureolysin	10–29, 46–56, 63–74, 83–105, 107–114, 138–145, 170–184, 186–193, 216–221, 242–248, 277–289, 303–311, 346–360, 379–389, 422–428, 446–453, 459–469, 479–489, 496–501	A:1, C: 6	aa 83–156	A:GSBXO46(83–156):14/29	10, 62

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
2359	ORF0409	lSSP, immuno-genic secreted protein precursor, putative	4-29, 92-99, 119-130, 228-236, 264-269, 271-280, 311-317, 321-331, 341-353, 357-363, 366-372, 377-384, 390-396, 409-415, 440-448, 458-470, 504-520, 544-563, 568-581, 584-592, 594-603, 610-616	B:4, F:11	aa 168-184 aa 206-220 aa 297-309	B:GSBXD01(168-184):1/1 B:GSBXD62(205-220):1/1 B:GSBXC17(297-309):6/27 F:SALAL04(205-220):9/41	9, 61
2378	ORF0398	SrpA	18-23, 42-55, 69-77, 85-98, 129-136, 182-188, 214-220, 229-235, 242-248, 251-258, 281-292, 309-316, 333-343, 348-354, 361-367, 393-407, 441-447, 481-488, 493-505, 510-515, 517-527, 530-535, 540-549, 564-583, 593-599, 608-621, 636-645, 656-670, 674-687, 697-708, 726-734, 755-760, 765-772, 785-792, 798-815, 819-824, 826-838, 846-852, 889-904, 907-913, 932-939, 956-964, 982-1000, 1008-1015, 1017-1024, 1028-1034, 1059-1065, 1078-1084, 1122-1129, 1134-1143, 1180-1186, 1188-1194, 1205-1215, 1224-1230, 1276-1283, 1333-1339, 1377-1382, 1415-1421, 1448-1459, 1467-1472, 1537-1545, 1556-1566, 1647-1654, 1666-1675, 1683-1689, 1722-1737, 1740-1754, 1756-1762, 1764-1773, 1775-1783, 1800-1809, 1811-1819, 1839-1851, 1859-1866, 1876-1882, 1930-1939, 1947-1954, 1978-1985, 1999-2007, 2015-2029, 2080-2086, 2094-2100, 2112-2118, 2196-2205, 2232-2243	C:1, D:7, F:4, H:11	aa 198-258 aa 646-727 aa 846-857 aa 2104-2206	C:GSBYI73(646-727): 2/9 F:SALAO33(846-857):10/41 D:n.d.	8, 60
2466	ORF0302	YycH protein	16-38, 71-77, 87-94, 105-112, 124-144, 158-164, 169-177, 180-186, 194-204, 221-228, 236-245, 250-267, 336-343, 363-378, 385-394, 406-412, 423-440, 443-449	D:14	aa 401-494	D:n.d.	7, 59
2470	ORF0299	Conserved hypo-thetical protein	4-9, 17-41, 50-56, 63-69, 82-87, 108-115, 145-151, 207-214, 244-249, 284-290, 308-316, 323-338, 348-358, 361-378, 410-419, 445-451, 512-522, 527-533, 540-546, 553-558, 561-575, 601-608, 632-644, 656-667, 701-713, 727-733, 766-780	C:3	aa 414-455	C:GSBYH60(414-455):28/31	169,170

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
2498	ORF0267	Conserved hypothetical protein	33–43, 45–51, 57–63, 65–72, 80–96, 99–110, 123–129, 161–171, 173–179, 185–191, 193–200, 208–224, 227–246, 252–258, 294–308, 321–329, 344–352, 691–707	D:12	aa 358–411 aa 588–606	D:17/21	6, 58
2548	ORF2711	IgG binding protein A	4–16, 24–57, 65–73, 85–91, 95–102, 125–132, 146–152, 156–163, 184–190, 204–210, 214–221, 242–252, 262–268, 272–279, 300–311, 320–337, 433–440, 472–480, 505–523	A:55, B:54, C:35, F:59, G:56, H:38	aa 1–48 aa 47–143 aa 219–285 aa 345–424	A:GSBXK68(1–73):21/30 A:GSBXK41(47–135):1/1 A:GSBXN38(219–285):19/30 A:GSBXL11(322–375):10/30 B:GSBXB22(406–418):37/71 F:SALAM17(406–418):29/41	53, 105
2577	ORF2683	Hypothetical protein	4–21, 49–56, 65–74, 95–112, 202–208, 214–235	C:6	aa 99–171	C:GSBYL56(99–171):1/1	149, 157
2642	ORF2614	unknown	34–58, 63–69, 74–86, 92–101, 130–138, 142–150, 158–191, 199–207, 210–221, 234–249, 252–271	C:1, E:1	aa 5–48	C:bhe3(5–48):25/30[#]	52, 104
2664	ORF2593	Conserved hypothetical protein	7–37, 56–71, 74–150, 155–162, 183–203, 211–222, 224–234, 242–272	D:35	aa 77–128	D:n.d.	51, 103
2670	ORF2588	Hexose transporter	18–28, 36–49, 56–62, 67–84, 86–95, 102–153, 180–195, 198–218, 254–280, 284–296, 301–325, 327–348, 353–390, 397–402, 407–414, 431–455	D:16	aa 328–394	D:n.d.	50, 102
2680	ORF2577	Coagulase	4–18, 25–31, 35–40, 53–69, 89–102, 147–154, 159–165, 185–202, 215–223, 284–289, 315–322, 350–363, 384–392, 447–453, 473–479, 517–523, 544–550, 572–577, 598–604, 617–623	C:26, G:4, H:8	aa 438–516 aa 505–570 aa 569–619	C:GSBYH16(438–516):3/5 C:GSBYG24(505–570):1/7 C:GSBYL82(569–619):2/7	148, 156
2740	ORF2515	Hypothetical protein	5–44, 47–55, 62–68, 70–78, 93–100, 128–151, 166–171, 176–308	D:4	aa 1–59	D:n.d.	49, 101
2746	ORF2507	homology with ORF1	5–12, 15–20, 43–49, 94–106, 110–116, 119–128, 153–163, 175–180, 185–191, 198–209, 244–252, 254–264, 266–273, 280–288, 290–297	A:1, H:13	aa 63–126	A:GSBXO40(66–123):8/29	48, 100
2797	ORF2470	unknown	10–27, 37–56, 64–99, 106–119, 121–136, 139–145, 148–178, 190–216, 225–249, 251–276, 292–297, 312–321, 332–399, 403–458	B:3, E:2, F:13, H:3	aa 183–200 aa 349–363	B:GSBXE85(183–200):11/27 F:SALAQ47(183–200):8/41	47, 99

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
2798	ORF2469	Lipase (geh)	12-35, 93-99, 166-179, 217-227, 239-248, 269-276, 288-294, 296-320, 322-327, 334-339, 344-356, 362-371, 375-384, 404-411, 433-438, 443-448, 455-464, 480-486, 497-503, 516-525, 535-541, 561-570, 579-585, 603-622, 633-641	A:41, B:42, C:3, F:35, G:1, H:11	aa 48-136 aa 128-172 aa 201-258	C:GSBYG01(48-136):2/6 A:GSBXM31-bmd12(128-188):11/30 B:GSBXE16(165-177):10/30 A:GSBXN20(201-258):8/30 F:SALAW05(165-177):13/41	46, 98
2815	ORF2451	Conserved hypothetical protein	5-32, 34-49	D:21	aa 1-43	D:n.d.	45, 97
2914	ORF2351	metC	39-44, 46-80, 92-98, 105-113, 118-123, 133-165, 176-208, 226-238, 240-255, 279-285, 298-330, 338-345, 350-357, 365-372, 397-402, 409-415, 465-473, 488-515, 517-535, 542-550, 554-590, 593-601, 603-620, 627-653, 660-665, 674-687, 698-718, 726-739	A:1, C:14, F:2	aa 386-402	A:GSBXM18(386-402):17/29	44, 96
2960	ORF2298	putative Exotoxin	13-36, 40-49, 111-118, 134-140, 159-164, 173-183, 208-220, 232-241, 245-254, 262-271, 280-286, 295-301, 303-310, 319-324, 332-339	C:101, E:2, H:58	aa 1-85 aa 54-121 aa 103-195	C:GSBYG32(1-85)::6/7 C:GSBYG61-bhe2(54-121):26/30 C:GSBYN80(103-195):13/17	43, 95
2963	ORF2295	putative Exotoxin	13-28, 40-46, 69-75, 86-92, 114-120, 126-137, 155-172, 182-193, 199-206, 213-221, 232-238, 243-253, 270-276, 284-290	C:3, E:3, G:1	aa 22-100	C:GSBYJ58(22-100):9/15	147, 155
3002	ORF1704	homology with ORF1	4-21, 28-40, 45-52, 59-71, 92-107, 123-137, 159-174, 190-202, 220-229, 232-241, 282-296, 302-308, 312-331	A:2, C:1, H:4	aa 21-118	A:GSBXL06(21-118):50/52	33, 85

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
3200	ORF1331	putative extracellular matrix binding protein	6-15, 22-32, 58-73, 82-88, 97-109, 120-131, 134-140, 151-163, 179-185, 219-230, 242-255, 271-277, 288-293, 305-319, 345-356, 368-381, 397-406, 408-420, 427-437, 448-454, 473-482, 498-505, 529-535, 550-563, 573-580, 582-590, 600-605, 618-627, 677-685, 718-725, 729-735, 744-759, 773-784, 789-794, 820-837, 902-908, 916-921, 929-935, 949-955, 1001-1008, 1026-1032, 1074-1083, 1088-1094, 1108-1117, 1137-1142, 1159-1177, 1183-1194, 1214-1220, 1236-1252, 1261-1269, 1289-1294, 1311-1329, 1336-1341, 1406-1413, 1419-1432, 1437-1457, 1464-1503, 1519-1525, 1531-1537, 1539-1557, 1560-1567, 1611-1618, 1620-1629, 1697-1704, 1712-1719, 1726-1736, 1781-1786, 1797-1817, 1848-1854, 1879-1890, 1919-1925, 1946-1953, 1974-1979	A:11, B:11, C:36	aa 5-134	A:GSBXL07(5-134):6/28	29, 81

Table 2b: Additional immunogenic proteins identified by bacterial surface and ribosome display: S. aureus

Bacterial surface display: A, LSA250/1 library in fhuA with patient sera 1 (655); B, LSA50/6 library in lamB with patient sera 1 (484); C, LSA250/1 library in fhuA with IC sera 1 (571); E, LSA50/6 library in lamB with IC sera 2 (454); F, LSA50/6 library in lamB with patient sera P1 (1105); G, LSA50/6 library in lamb with IC sera 1 (471); H, LSA250/1 library in fhuA with patient sera 1 (IgA, 708). Ribosome display: D, LSA250/1 library with IC sera (1686). **, prediction of antigenic sequences longer than 5 amino acids was performed with the programme ANTIGENIC (Kolaskar and Tongaonkar, 1990). ORF, open reading frame; CRF, reading frame on complementary strand; ARF, alternative reading frame.

S. aureus antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ARF0280	Putative protein	7–14	F:6	aa 25–43	SALAM59(25–43): 1/1	401, 402
CRF0145	Putative protein	18–28, 31–37, 40–47, 51–83, 86–126	F:5	aa 81–90	SALAZ40(81–90): 2/12	403, 404
CRF0250	Putative protein	4–24, 26–46, 49–86	G:8	aa 60–76	SALAJ87(60–76): n.d.	365, 378
CRF0308	Putative protein	40–46	A:6, B:2, C:47, E:35	aa 5–38	A:GSBXK03(7–36):28/69 B:GSBXD29(10–20):10/27	391, 392
CRF0337	Unknown	4–17	D:3	aa 1–20	D:n.d.	469; 486
CRF0497	Putative protein	4–28, 31–53, 58–64	B:13, F:5	aa 18–34	GSBXF31(19–34): 1/7	366, 379
CRF0538	Unknown	4–20	D: 7	aa 1–11	D:n.d.	470; 487
CRF0750	Putative protein	4–11, 18–24, 35–40	G:44	aa 25–39	SALAG92(26–39): n.d.	367, 380
CRF1145	Unknown	4–57	D:28	aa 16–32	D:n.d.	464; 481
CRF1247	Putative protein	4–25, 27–56	F:6	aa 36–46	SALAR23(36–46): n.d.	368, 381
CRF1256	Putative protein	19–25, 38–47, 55–74, 77–87	G:5	aa 54–67	SALAG65(54–67): n.d.	369, 382
CRF1356	Unknown	8–15; 18–24; 27–38	D: 5	aa 5–33	D:n.d.	471; 488
CRF1763	Putative protein	4–9, 23–41, 43–58, 71–85	C:3	aa 1–22	C:GSBYI30(1–22):1/1	407, 408
CRF1783	Unknown	8–161	D: 5	aa 76–127	D:n.d.	465; 482
CRF1845	Unknown	4–28; 30–36	D: 272	aa 1–17	D:n.d.	472; 489
CRF1861	Unknown	6–11; 13–34; 36–50	D:8	aa 4–27	D:n.d.	466; 483
CRF1928	Putative protein	4–9, 17–30	F:9	aa 13–22	SALAR41(13–22): n.d.	370, 383
CRF2004	Putative protein	18–38	F:13	aa 16–32	SALAM75(16–32): n.d.	371, 384
CRF2155	Putative protein	4–15, 30–58	F:9	aa 54–66	SALAQ54(54–66):1/12	372, 385
CRF2180	Putative protein	4–61, 65–72, 79–95, 97–106	E:13	aa 86–99	GSBZE08(86–99): n.d.	373, 386
CRF2207	Unknown	4–13	D: 3	aa 17–39	D:n.d.	473; 490
CRF2305	Putative protein	4–9, 22–33, 44–60	C:5	aa 80–116	GSBYL75(80–116): n.d.	374, 387
CRF2341	Putative protein	4–23, 30–44, 49–70	F:8	aa 46–55	SALAW31(46–55): n.d.	375, 388
CRF2349	Putative protein	4–32, 39–46, 62–69, 77–83	B:10, F:4	aa 46–67	GSBXC92(52–67):2/11	376, 389

S. aureus antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
CRF2356	Unknown	4–18	D: 3	aa 3–18	D:n.d.	475; 492
CRF2452	Unknown	4–31	D: 9	aa 7–21	D:n.d.	476; 493
CRF2498	Putative protein	4–29, 31–41	G:8	aa 2–15	SALAF30(3–15): n.d.	377, 390
CRF2553	Unknown	4–35; 37–42	D: 4	aa 1–20	D:n.d.	474; 491
CRF2578	Unknown	5–25; 30–39	D: 11	aa 9–30	D:n.d.	467; 484
CRF2664	Unknown	11–21	D: 17	aa 1–14	D:n.d.	477; 494
CRF2729	Putative protein	10–41, 50–57	F:3	aa 40–56	SALAQ25(40–56): 1/1	405, 406
CRF2863/1	Unknown	4–43	D: 78	aa 17–40	D:n.d.	478; 495
CRF2863/2	Unknown	4–46	D: 78	aa 44–49	D:n.d.	479; 496
CRFA002	Unknown	17–39;52–59	D: 3	aa 38–55	D:n.d.	463; 480
CRFN1	Unknown	5–20; 37–44; 52–59; 87–94; 116–132	D: 4	aa 94–116	D:n.d.	468; 485
ORF0188	UDP–N–acetyl–D–mannosamine transferase, putative	11–18, 43–56, 58–97, 100–118, 120–148, 152–171, 195–203, 207–214, 220–227, 233–244	B:4, F:29	aa 197–210	SALAM14(198–209): n.d.	397, 398
ORF0254	Multidrug efflux transporter	4–33, 35–56, 66–99, 109–124, 136–144, 151–180, 188–198, 201–236, 238–244, 250–260, 266–290, 294–306, 342–377	D: 3	aa 155–175	D: n.d.	297,325
ORF0307	Conserved hypothetical protein	4–23, 25–67, 76–107, 109–148	D: 3	aa 9 – 44	D: n.d.	298, 326
ORF0452	Conserved hypothetical protein	4–35, 41–47, 55–75, 77–89, 98–113, 116–140, 144–179, 194–215, 232–254, 260–273, 280–288, 290–302, 315–323, 330–369, 372–385, 413–432	D: 5	aa 105–122	D: n.d.	299, 327
ORF0456	Na+/H+Antiporter	4–81	D: 66	aa 1–21	D: n.d.	300, 328
ORF0556	Iron(III)dicitrate binding protein	5–23, 50–74, 92–99, 107–122, 126–142, 152–159, 172–179, 188–196, 211–218, 271–282	D: 10	aa 1–18	D: n.d.	301, 329
ORF0629	Hypothetical Protein	9–44, 63–69, 75–82, 86–106, 108–146, 153–161, 166–178, 185–192, 233–239, 258–266, 302–307	D: 313	aa 13 – 37	D: n.d.	302, 330

S. aureus antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF0637	GTP-binding protein TypA	10-19, 22-32, 95-105, 112-119, 121-133, 140-154, 162-174, 186-200, 207-224, 238-247, 254-266, 274-280, 288-294, 296-305, 343-351, 358-364, 366-373, 382-393, 403-413, 415-422, 440-447, 499-507, 565-575, 578-588	F:3	aa 107-119	F:SALAX70(107-119):10/41	393, 395
ORF0713	Conserved hypothetical transmembrane protein, putative	22-51, 53-71, 80-85, 93-99, 105-112, 123-146, 151-157, 165-222, 226-236, 247-270, 290-296, 301-324, 330-348, 362-382, 384-391, 396-461, 463-482, 490-515	D: 3	aa 487 – 513	D: n.d.	303, 331
ORF0788	Cell division protein	104-111, 158-171, 186-197, 204-209, 230-247, 253-259, 269-277, 290-314, 330-340, 347-367, 378-388	D: 4	aa 152 – 178	D: n.d.	304, 332
ORF0797	Conserved hypothetical protein	11-40, 56-75, 83-102, 112-117, 129-147, 154-168, 174-191, 196-270, 280-344, 354-377, 380-429, 431-450, 458-483, 502-520, 525-532, 595-602, 662-669, 675-686, 696-702, 704-711, 720-735, 739-748, 750-756, 770-779, 793-800, 813-822, 834-862	D:12	aa 196 –218	D: n.d.	305, 333
ORF0836	Cell Division Protein	34-91, 100-119, 126-143, 147-185, 187-197, 319-335, 349-355, 363-395, 397-412, 414-422, 424-440, 458-465, 467-475, 480-505, 507-529, 531-542, 548-553, 577-589, 614-632, 640-649, 685-704, 730-741, 744-751, 780-786	D:5	aa 26 – 56	D: n.d.	306, 334
ORF1318	Amino acid permease	11-21, 25-32, 34-54, 81-88, 93-99, 105-117, 122-145, 148-174, 187-193, 203-218, 226-260, 265-298, 306-318, 325-381, 393-399, 402-421, 426-448	D: 8	aa127 – 152	D: n.d.	307, 335
ORF1321	Pyruvat kinase	4-11, 50-67, 89-95, 103-109, 112-135, 139-147, 158-170, 185-204, 213-219, 229-242, 248-277, 294-300, 316-323, 330-335, 339-379, 390-402, 408-422, 431-439, 446-457, 469-474, 484-500, 506-513, 517-530, 538-546, 548-561	E:6	aa 420-432	E:GSBZE16(420-432):5/41	197, 216

S. aureus antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF1388	LPXTG cell wall anchor motif	11-31, 86-91, 103-111, 175-182, 205-212, 218-226, 242-247, 260-269, 279-288, 304-313, 329-334, 355-360, 378-387, 390-399, 407-435, 468-486, 510-516, 535-547, 574-581, 604-615, 635-646, 653-659, 689-696, 730-737, 802-812, 879-891, 893-906, 922-931, 954-964, 997-1009, 1031-1042, 1089-1096, 1107-1120, 1123-1130, 1149-1162, 1176-1184, 1192-1207, 1209-1215, 1253-1259, 1265-1275, 1282-1295, 1304-1310, 1345-1361, 1382-1388, 1394-1400, 1412-1430, 1457-1462, 1489-1507, 1509-1515, 1535-1540, 1571-1591, 1619-1626, 1635-1641, 1647-1655, 1695-1701, 1726-1748, 1750-1757, 1767-1783, 1802-1807, 1809-1822, 1844-1875, 1883-1889, 1922-1929, 1931-1936, 1951-1967, 1978-1989, 1999-2008, 2023-2042, 2056-2083, 2101-2136, 2161-2177	D: 3	aa 508 - 523	D: n.d.	308, 336
ORF1402	3,4-dihydroxy-2-butanone-4-phosphate synthase	18-23, 32-37, 54-63, 65-74, 83-92, 107-114, 123-139, 144-155, 157-164, 191-198, 232-240, 247-272, 284-290, 295-301, 303-309, 311-321, 328-341, 367-376	E:3	aa 121-137	E:GSBZB68(121-137):7/41	198, 217
ORF1473	hemolysin II (LukD-Leuktoxin)	4-36, 39-47, 57-65, 75-82, 108-114, 119-126, 135-143, 189-195, 234-244, 250-257, 266-272, 311-316	F:1	aa 245-256	F:SALAP76(245-256):6/41	199, 218
ORF1523	Iron uptake regulator	13-27, 29-44, 46-66, 68-81, 97-116, 138-145	D:3	aa 120- 135	D: n.d.	309, 337
ORF1707	inner membrane protein, 60 kDa	4-23, 57-77, 89-103, 119-125, 132-172, 179-197, 210-254, 256-265, 281-287	F :1	aa 104-118	F:SALBC82(104-118):7/41	200, 219
ORF1754	amiB	5-10, 16-24, 62-69, 77-96, 100-115, 117-126, 137-156, 165-183, 202-211, 215-225, 229-241, 250-260, 267-273, 290-300, 302-308, 320-333, 336-342, 348-356, 375-382, 384-389	D: 3	aa 293 - 312	D: n.d.	310, 338

S. aureus antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF1783	Mrp protein (fmtB)	5-29, 46-52, 70-76, 81-87, 155-170, 192-197, 206-213, 215-220, 225-231, 249-258, 273-279, 281-287, 300-306, 313-319, 323-332, 335-341, 344-351, 360-382, 407-431, 443-448, 459-468, 475-496, 513-520, 522-537, 543-550, 556-565, 567-573, 580-585, 593-615, 619-631, 633-642, 670-686, 688-698, 759-766, 768-782, 799-808, 842-848, 868-877, 879-917, 945-950, 979-988, 996-1002, 1025-1036, 1065-1084, 1101-1107, 1113-1119, 1125-1142, 1163-1169, 1183-1189, 1213-1219, 1289-1301, 1307-1315, 1331-1342, 1369-1378, 1385-1391, 1410-1419, 1421-1427, 1433-1447, 1468-1475, 1487-1494, 1518-1529, 1564-1570, 1592-1609, 1675-1681, 1686-1693, 1714-1725, 1740-1747, 1767-1774, 1793-1807, 1824-1841, 1920-1937, 1953-1958, 1972-1978, 1980-1986, 1997-2011, 2048-2066, 2161-2166, 2219-2224, 2252-2257, 2292-2298, 2375-2380, 2394-2399, 2435-2440, 2449-2468	F:2	aa 850-860	F:SALAQ36(850-860):8/41	201, 220
ORF1848	Map-ND2C protein	4-27, 42-66, 70-76, 102-107, 113-118, 133-138	E:5	aa 75-90	E:GSBZB15(75-90):6/41	202, 221
ORF1891	ribosomal protein L2 (rplB)	31-39, 48-54, 61-67, 75-83, 90-98, 103-119, 123-145, 160-167, 169-176, 182-193, 195-206, 267-273	F:4	aa 239-257	F:SALAV36(239-257):19/41	203, 222
ORF2011	Putative drug transporter	5-27, 79-85, 105-110, 138-165, 183-202, 204-225, 233-259, 272-292, 298-320, 327-336, 338-345, 363-376, 383-398, 400-422, 425-470, 489-495, 506-518, 536-544, 549-554, 562-568, 584-598, 603-623	D:5	aa 205 - 224	D: n.d.	311, 339
ORF2027	lactase permease, putative	10-33, 38-71, 73-103, 113-125, 132-147, 154-163, 170-216, 222-248, 250-269, 271-278, 287-335, 337-355, 360-374, 384-408, 425-442, 453-465, 468-476, 478-501, 508-529	E:2	aa 422-436	E:GSBZF58(422-436):6/41	204, 223
ORF2087	Hemolysin II (putative)	8-27, 52-59, 73-80, 90-99, 104-110, 117-124, 131-140, 189-209, 217-232, 265-279, 287-293, 299-306	D: 3	aa 126 - 147	D: n.d.	312, 340

S. aureus antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2090	preLukS	8-26, 75-82, 118-126, 136-142, 163-177, 182-189, 205-215, 221-236, 239-248, 268-274	F:2	aa 270-284	F:SALAQ77(270-284):23/41	205, 224
ORF2092	Hemolysin II (preLUK-F)	5-22, 30-47, 58-65, 75-81, 87-92, 99-105, 107-113, 119-126, 189-195, 217-223, 234-244, 250-257, 266-272	F:3	aa 238-253	F:SALAQ67(237-252):10/41	206, 225
ORF2107	Multidrug resistance protein (putative)	10-28, 30-43, 50-75, 80-113, 116-125, 136-167, 170-191, 197-245, 253-329, 345-367, 375-396	D: 9	aa 54 – 104	D: n.d.	313, 341
ORF2192	Transcriptional regulator GntR family, putative	20-31, 46-52, 55-69, 74-79, 89-97, 108-113, 120-128, 141-171, 188-214	D: 3	aa 15 – 35	D: n.d.	314, 342
ORF2305	Amino acid permease	25-79, 91-103, 105-127, 132-149, 158-175, 185-221, 231-249, 267-293, 307-329, 336-343, 346-359, 362-405, 415-442, 446-468	D: 53	aa 363 – 393	D: n.d.	315, 343
ORF2324	Citrate dransporter	10-77, 85-96, 99-109, 111-138, 144-155, 167-176, 178-205, 225-238, 241-247, 258-280, 282-294, 304-309, 313-327, 333-383, 386-402, 405-422, 429-453	D: 7	aa 37 – 83	D: n.d.	316, 344
ORF2422	Anion transporter family protein	7-26, 28-34, 36-53, 55-73, 75-81, 87-100, 108-117, 121-138, 150-160, 175-181, 184-195, 202-215, 221-247, 265-271, 274-314, 324-337, 341-412, 414-423, 425-440, 447-462, 464-469	D: 16	aa 275 – 295	D: n.d.	317, 345
ORF2553	SirA	5-22, 54-78, 97-103, 113-123, 130-148, 166-171, 173-180, 192-201, 254-261, 266-272, 310-322	D:3	aa 1 – 22	D: n.d.	318, 346
ORF2555	ornithine cyclode-aminase	20-35, 37-50, 96-102, 109-120, 123-137, 141-150, 165-182, 206-224, 237-256, 267-273, 277-291, 300-305, 313-324	E:2	aa 32-48	E:GSBZB37(32-48):11/41	207, 226
ORF2558	Multidrug resistance efflux protein, putative	11-63, 79-129, 136-191, 209-231, 237-250, 254-276, 282-306, 311-345, 352-373, 376-397	D: 8	aa 84 – 100	D: n.d.	319, 347
ORF2610	Cap5M	4-30, 34-40, 79-85, 89-98, 104-118, 124-139, 148-160, 167-178	D: 13	aa 114 – 141	D: n.d.	320, 348
ORF2613	Cap5P (UDP-N-acetylglucosamine 2-epimerase)	4-9, 17-24, 32-38, 44-54, 68-82, 89-95, 101-120, 124-131, 136-142, 145-157, 174-181, 184-191, 196-204, 215-224, 228-236, 243-250, 259-266, 274-281, 293-301, 314-319, 325-331, 355-367, 373-378	B:3, F:11	aa 321-341	F:SALAU27(325-337):9/41	208, 227

S. aureus antigenic protein	Putative function (by homology)	predicted Immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2628	Hypothetical protein	9–15, 28–36, 44–62, 69–88, 98–104, 111–136, 139–149, 177–186, 195–217, 224–236, 241–257, 260–278, 283–290, 292–373, 395–408, 411–443, 465–472, 475–496, 503–520, 552–559, 569–589, 593–599, 607–613, 615–636, 648–654, 659–687, 689–696, 721–733, 738–759, 783–789, 795–801, 811–823, 827–836, 839–851, 867–875, 877–883, 890–898, 900–908, 912–931, 937–951, 961–992, 994–1002, 1005–1011, 1016–1060, 1062–1074, 1088–1096, 1101–1123, 1137–1153, 1169–1192, 1210–1220, 1228–1239, 1242–1251, 1268–1275, 1299–1311, 1322–1330, 1338–1361, 1378–1384, 1393–1412, 1419–1425, 1439–1459, 1469–1482, 1489–1495, 1502–1519, 1527–1544, 1548–1555, 1600–1607, 1609–1617, 1624–1657, 1667–1691, 1705–1723, 1727–1742, 1749–1770, 1773–1787, 1804–1813, 1829–1837, 1846–1852, 1854–1864, 1869–1879, 1881–1896, 1900–1909, 1922–1927, 1929–1935, 1942–1962, 1972–2005, 2009–2029, 2031–2038, 2055–2076, 2101–2114, 2117–2124, 2147–2178, 2188–2202, 2209–2217, 2224–2230, 2255–2266, 2271–2280, 2282–2302, 2307–2316, 2319–2324, 2379–2387	F:6	aa 694–708 aa 790–800 aa 1288–1305	F:SALBD82(1288–1303):9/41	209, 228
ORF2644	PTS system, sucrose–specific IIBC component	8–15, 24–30, 49–68, 80–93, 102–107, 126–147, 149–168, 170–180, 185–193, 241–305, 307–339, 346–355, 358–372, 382–390, 392–415, 418–425, 427–433, 435–444, 450–472	F:4	aa 106–159	F:SALAW60(106–125):3/41	210, 229
ORF2654	Oligopeptide ABC transporter, putative	5–61, 72–84, 87–99, 104–109, 124–145, 158–170, 180–188, 190–216, 223–264, 270–275, 296–336, 355–372	D: 5	aa 182 –209	D: n.d.	321, 349
ORF2662	maltose ABC transporter, putative	4–21, 71–79, 99–105, 110–121, 143–161, 199–205, 219–235, 244–258, 265–270, 285–291, 300–308, 310–318, 322–328, 346–351, 355–361, 409–416	F:1	aa 306–323	F:SALBC05(306–323):2/41	211, 230

S. *aureus* antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2710	sorbitol dehydrogenase	4-12, 19-40, 61-111, 117-138, 140-153, 161-180, 182-207, 226-235, 237-249, 253-264, 267-274, 277-292, 311-323	B:2, F:4	aa 244-257	F:SALAX93(249-256):6/41	212, 231
ORF2742	Hypothetical protein	4-41, 49-56, 61-67, 75-82, 88-104, 114-125, 129-145, 151-165, 171-178, 187-221, 224-230, 238-250, 252-275, 277-304, 306-385	D: 188, H:4	aa 303 - 323	D: n.d.	322, 350
ORF2780	bmQ	4-29, 41-63, 74-95, 97-103, 107-189, 193-209, 220-248, 260-270, 273-299, 301-326, 328-355, 366-397, 399-428	D: 3	aa 26 - 40	D: n.d.	323, 351
ORF2806	Phage related protein	10-17, 23-29, 31-37, 54-59, 74-81, 102-115, 127-137, 145-152, 158-165, 178-186, 188-196, 203-210, 221-227, 232-237	F:3	aa 104-116	F:SALBC34:1/1	213, 232
ORF2900	Conserved hypothetical protein	4-27, 34-43, 62-73, 81-90, 103-116, 125-136, 180-205, 213-218, 227-235, 238-243, 251-259, 261-269, 275-280, 284-294, 297-308, 312-342, 355-380, 394-408, 433-458, 470-510, 514-536, 542-567	D: 24	aa 360 - 376	D: n.d.	324, 352
ORF2931	conserved hypothetical protein	4-19, 43-54, 56-62, 84-90, 96-102, 127-135, 157-164, 181-187	E:6	aa 22-37	E:GSBZA13(22-37):7/41	214, 233
ORF2958	Exotoxin 2	7-19, 26-39, 44-53, 58-69, 82-88, 91-107, 129-141, 149-155, 165-178, 188-194	F:1	aa 154-168	F:SALBB59(154-168):4/41	215, 234
ORF2970	Surface protein, putative	9-23, 38-43, 55-60, 69-78, 93-101, 103-112, 132-148, 187-193, 201-208, 216-229, 300-312, 327-352, 364-369, 374-383, 390-396, 402-410, 419-426, 463-475, 482-491	H:5	aa 1-70	H:GSBYU66: n.d.	399, 400

Table 2c: Immunogenic proteins identified by bacterial surface and ribosome display: S. epidermidis.

Bacterial surface display: A, LSE150 library in fhuA with patient sera 2 (957); B, LSE70 library in lamB with patient sera 2 (1420); C, LSE70 library in lamB with patient sera 1 (551). Ribosome display: D, LSE150 in pMAL4.31 with P2 (1235). **, prediction of antigenic sequences longer than 5 amino acids was performed with the programme ANTIGENIC (Kolaskar and Tongaonkar,

1990). ORF, open reading frame; ARF, alternative reading frame; CRF, reading frame on complementary strand. ORF, open reading frame; CRF, reading frame on complementary strand.

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ARF0172	cation-transport-ing ATPase, E1-E2 family	4-34, 37-43	D:6	aa3-32	D: nd	497, 548
ARF0183	condensing en-zyme, putative, FabH-related	4-22, 24-49	D:4	aa1-52	D: nd	498, 549
ARF2455	NADH dehydrogenase, putative	4-29	D:3	aa1-22	D: nd	499, 550
CRF0001	Unknown	4-14, 16-26	D:3	aa5-21	D: nd	500, 551
CRF0002	Unknown	4-13, 15-23, 36-62	D:5	aa21-70	D: nd	501, 552
CRF0003	Unknown	4-12, 14-28	D:3	aa 4-31	D: nd	502, 553
CRF0004	Unknown	5-15, 35-71, 86-94	D:4	aa31-72	D: nd	503, 554
CRF0005	Unknown	8-26, 28-34	D:3	aa:9-33	D: nd	504, 555
CRF0006	Unknown	4-11, 15-28	D:3	aa10-22	D: nd	505, 556
CRF0007	Unknown	4-19, 30-36	D:3	aa 7-44	D: nd	506, 557
CRF0008	Unknown	10-48	D:4	aa:9-44	D: nd	507, 558
CRF0009	Unknown	41883	D:3	aa5-14	D: nd	508, 559
CRF0192	Putative protein	4-23, 25-68	C:4	aa 15-34	C:GSBBM10(15-34): n.d.	445, 446

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
CRF0275	Putative protein	4-40, 49-65	B:5	aa 35-68	B:SELAK28(35-68): n.d.	447, 448
CRF0622	Putative protein	4-12, 17-57, 62-70, 75-84, 86-100	C:4	aa 75-99	C:GSBBR74(76-99): n.d.	449, 450
CRF0879	Putative protein	4-14, 38-44	A:3, B:10	aa 9-40	B:SELAC39(10-40): n.d.	451, 452
CRF1004	Putative protein	4-40	A:3, B:5	aa 29-65	B:SELAI63(35-63): n.d.	453, 454
CRF2248	Putative protein	4-10, 19-40, 53-64, 74-91	C:30	aa 74-111	C:GSBBN64(16-35): n.d.	455, 456
CRF2307	Putative protein	4-19, 35-41, 80-89	A:19	aa 41-87	A:SEFAL47(41-87):n.d.	457, 458
CRF2309	Putative protein	15-21	B:6	aa 4-16	B:SELAL02(4-16): n.d.	459, 460
CRF2409	Putative protein	6-25	B:6	aa 2-24	B:SELAB48(5-24): n.d.	461, 462
ORF0005	hypothetical protein	13-27, 33-67, 73-99, 114-129, 132-158, 167-190, 193-234, 237-267, 269-299, 316-330, 339-351, 359-382, 384-423	D:3	aa105-128	D: nd	509, 560
ORF0008	Streptococcal hemagglutinin	9-14, 16-24, 26-32, 41-50, 71-79, 90-96, 177-184, 232-237, 271-278, 293-301, 322-330, 332-339, 349-354, 375-386, 390-396, 403-409, 453-459, 466-472, 478-486, 504-509, 518-525, 530-541, 546-552, 573-586, 595-600, 603-622, 643-660, 668-673, 675-681, 691-697, 699-711, 713-726, 732-749, 753-759, 798-807, 814-826, 831-841, 846-852, 871-878, 897-904, 921-930, 997-1003, 1026-1031, 1033-1039, 1050-1057, 1069-1075, 1097-1103, 1105-1111, 1134-1139, 1141-1147, 1168-1175, 1177-1183, 1205-1211, 1213-1219, 1231-1237, 1241-1247, 1267-1273, 1304-1309, 1311-1317, 1329-1335, 1339-1345, 1347-1353, 1382-1389, 1401-1407, 1411-1417, 1447-1453, 1455-1461, 1483-1489, 1491-1497, 1527-1533, 1545-1551, 1556-1561, 1581-1587, 1591-1597, 1627-1638, 1661-1667, 1684-1689, 1691-1697, 1708-1715, 1719-1725, 1765-1771, 1813-1820, 1823-1830, 1835-1856	B:2	aa 895-926	B:SELAF79(895-926): 7/12	239, 268

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF0038	extracellular elastase precursor	6-25, 29-35, 39-45, 64-71, 82-88, 96-102, 107-113, 119-131, 170-176, 186-192, 196-202, 215-220, 243-248, 302-312, 345-360, 362-371, 378-384, 458-470, 478-489, 495-504	C:6	aa 136-165	C:GSBBN08(136-165):1/1	353,359
ORF0099	hypothetical protein	6-18, 31-37, 42-49, 51-67, 73-85, 87-93, 102-109, 119-126, 150-157, 170-179; 185-191, 204-214, 217-223, 237-248, 269-275, 278-316, 320-340, 359-365	D:5	aa218-265	D: nd	510, 561
ORF0101	hypothetical protein	4-10, 15-27, 67-94, 123-129, 167-173, 179-184, 187-198, 217-222, 229-235, 238-246	D:18	aa26-109	D: nd	511, 562
ORF0121	C4-dicarboxylate transporter, an-aerobic, putative	4-20, 24-62, 73-86, 89-106, 110-122, 131-164, 169-193, 204-213, 219-236, 252-259, 263-281, 296-306, 318-324, 328-352, 356-397, 410-429	D:5	aa323-379	D: nd	512, 563
ORF0143	amino acid per-mease	25-79, 91-103, 105-127, 132-150, 157-174, 184-206, 208-219, 231-249, 267-294, 310-329, 336-343, 346-405, 417-468	D:35	aa247-339	D: nd	513, 564
ORF0162	Immunodominant Antigen A	4-27, 35-45, 52-68, 83-89, 113-119, 133-150, 158-166, 171-176, 198-204, 219-230	A:11, B:11; C:153	aa 90-227	B:SELAA19(100-118): 1/1 B:SELAE24(170-190): 11/12	240, 269
ORF0201	capa protein, putative	10-17, 27-53, 81-86, 98-105, 126-135, 170-176, 182-188, 203-217, 223-232, 246-252, 254-269, 274-280, 308-314	D:9	aa11-53	D: nd	514, 565
ORF0207	Ribokinase (rbsK)	5-11, 15-23, 47-55, 82-90, 98-103, 108-114, 126-132, 134-156, 161-186, 191-197, 210-224, 228-235, 239-248, 258-264, 275-290	B:10	aa 20-45	B:SELAQ30 (20-45): 12/12	241, 270
ORF0288	LrgB	7-28, 34-56, 68-119, 127-146, 149-180, 182-189, 193-200, 211-230	D:4	aa112-149	D: nd	515, 566

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF0304	Herpesvirus saimiri ORF73 homolog, putative	8-16, 30-36, 83-106, 116-122, 135-143, 152-165, 177-188, 216-225	D:8	aa69-117	D: nd	516, 567
ORF0340	nitrate transporter	7-21, 24-93, 101-124, 126-139, 141-156, 163-179, 187-199, 202-242, 244-261, 267-308, 313-322, 340-353, 355-376	D:5	aa238-309	D: nd	517, 595
ORF0346	hypothetical protein	8-27, 65-73, 87-93, 95-105	D:8	aa 1-29	D: nd	518, 568
ORF0355	conserved hypothetical protein	5-30, 37-43, 57-66, 85-94, 103-111, 118-125	C:5	aa 63-86	C:GSBBL39(63-86):1/1	354, 360
ORF0356	conserved hypothetical protein	4-14, 21-53, 60-146, 161-173, 175-182, 190-198, 200-211	D:5	aa51-91	D: nd	519, 569
ORF0406	hypothetical protein	12-32, 35-63, 68-102, 106-137, 139-145, 154-168, 173-185, 203-222, 230-259, 357-364, 366-374	D:19	aa1-48, aa69-102	D: nd	520, 570
ORF0425	amino acid permease	40-58, 75-86, 93-110, 117-144, 150-173, 199-219, 229-260, 264-300, 317-323, 329-356, 360-374, 377-390, 392-398, 408-424, 427-452	D:3	aa401-440	D: nd	521, 571
ORF0442	SceB precursor	7-22, 42-48, 55-66, 83-90, 109-118, 136-141	C:38	aa 60-102	C:GSBBM60(65-84):1/1	355, 361
ORF0448	SsaA precursor	6-25, 39-47, 120-125, 127-135, 140-148, 157-168, 200-208, 210-220, 236-243, 245-254	C:170	aa 15-208	C:GSBBN58(81-105):1/1 C:GSBBL13(167-184):1/1 C:GSBBL25(22-45):1/1	356, 362
ORF0503	Ribosomal protein L2	31-39, 48-54, 61-67, 75-83, 90-98, 103-115, 123-145, 160-167, 169-176, 182-193, 195-206, 267-273	A:1, B:3	aa 212-273	B:SELAA47(238-259):12/12	242, 271
ORF0551	Conserved hypothetical protein	5-25, 29-36, 45-53, 62-67, 73-82, 84-91, 99-105, 121-142, 161-177, 187-193, 203-224, 242-251, 266-271, 278-285	A:16, B:9	aa 162-213	B:SELAL12(164-197): 8/12	243, 272
ORF0556	hypothetical protein	4-24, 30-41, 43-68, 82-90, 107-114, 123-143, 155-168	D:3	aa 1-26	D: nd	522, 596

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF0623	Fumble, putative	10-17, 32-38, 55-72, 77-84, 88-96, 126-134, 152-160, 176-185, 190-203, 208-214, 217-225, 233-252, 257-262	A:10, B:12; C:1	aa 95-150	B:SELAB86(95-128): 3/12	244, 273
ORF0740	Hypothetical protein	18-24, 47-61, 69-83, 90-96, 125-132, 140-163, 171-188, 222-249, 281-296, 305-315, 322-330, 335-351, 354-368, 390-397, 411-422, 424-431, 451-469, 479-485, 501-507, 517-524, 539-550, 560-568, 588-599, 619-627, 662-673, 678-689, 735-742, 744-749, 780-786, 797-814, 821-827, 839-847, 857-863, 866-876, 902-911, 919-924, 967-982, 1005-1015, 1020-1026, 1062-1070, 1078-1090, 1125-1131, 1145-1150, 1164-1182, 1208-1213, 1215-1234, 1239-1251, 1256-1270, 1298-1303, 1316-1325, 1339-1349, 1362-1369, 1373-1384, 1418-1427, 1440-1448, 1468-1475, 1523-1532, 1536-1542, 1566-1573, 1575-1593, 1603-1619, 1626-1636, 1657-1667, 1679-1687, 1692-1703, 1711-1718, 1740-1746, 1749-1757, 1760-1769, 1815-1849, 1884-1890, 1905-1914, 1919-1925, 1937-1947, 1955-1963, 1970-1978, 2003-2032, 2075-2089, 2117-2124, 2133-2140, 2146-2151, 2161-2167, 2173-2179, 2184-2196, 2204-2220, 2244-2254, 2259-2264, 2285-2296, 2300-2318, 2328-2334, 2347-2354, 2381-2388, 2396-2408, 2419-2446, 2481-2486, 2493-2500, 2506-2516, 2533-2540, 2555-2567, 2576-2592, 2599-2606, 2615-2639, 2647-2655	B:3	aa 1093-1114	B:SELAB23(1097-1114): 7/12	245, 274
ORF0757	hypothetical protein	13-20, 22-28, 33-40, 60-76, 79-86, 90-102, 112-122, 129-147, 157-170, 178-185, 188-193, 200-205, 218-228, 234-240, 243-250, 265-273, 285-291, 310-316, 330-348, 361-380, 399-405, 427-446, 453-464	C:6	aa 260-284	C:GSBBN01(260-284):1/1	357, 363

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF0912	DNA mismatch repair protein	9–16, 28–39, 47–56, 69–76, 104–121, 124–130, 137–144, 185–195, 199–214, 238–243, 293–307, 317–337, 351–370, 385–390, 411–428, 472–488, 498–516, 518–525, 528–535, 538–545, 553–559, 563–568, 579–588, 592–607, 615–622, 632–638, 641–648, 658–674, 676–705, 709–720, 727–739, 742–750, 753–760, 768–773, 783–788, 811–819, 827–838	A:25	aa 242–304	SEFAT31(242–290): n.d.	441, 442
ORF0923	GTP–binding protein	4–10, 18–27, 42–55, 64–72, 77–92, 114–126, 132–157, 186–196, 206–217, 236–243, 257–280, 287–300, 306–312, 321–328, 338–351, 360–367, 371–382, 385–399	B:13	aa 144–163	B:SELAD55(151–163): 8/12	246, 275
ORF0979	Conserved hypo–thetical protein	4–28, 44–51, 53–84, 88–107, 113–192	A:9, B:18	aa 12–51	B:SELAH01(26–49):5/12	247, 276
ORF0982	sodium/alanine symporter (alsT)	13–21, 24–50, 73–84, 91–118, 126–133, 142–149, 156–175, 189–249, 251–273, 294–332, 339–347, 358–381, 393–413, 425–448, 458–463	D:3	aa277–305	D: nd	523, 572
ORF1230	Signal peptidase I	6–33, 44–59, 61–69, 74–82, 92–98, 133–146, 163–175	D:14	aa 1–53	D: nd	524, 573
ORF1232	Exonuclease RexA	4–12, 16–32, 36–48, 50–65, 97–127, 136–142, 144–165, 176–190, 196–202, 211–222, 231–238, 245–251, 268–274, 280–286, 305–316, 334–356, 368–376, 395–402, 410–417, 426–440, 443–449, 474–486, 499–508, 510–525, 540–549, 568–576, 608–617, 624–639, 646–661, 672–678, 688–703, 706–717, 727–734, 743–755, 767–773, 783–797, 806–814, 830–839, 853–859, 863–871, 877–895, 899–918, 935–948, 976–990, 998–1007, 1020–1030, 1050–1062, 1070–1077, 1111–1125, 1137–1149, 1153–1160, 1195–1211	B:6	aa 188–219	B:SELAA13(188–216): n.d.	443, 444
ORF1284	permease PerM, putative	10–60, 72–96, 103–109, 127–133, 146–177, 182–189, 196–271, 277–289, 301–319, 323–344, 347–354	D:27	aa55–106	D: nd	525, 574

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF1319	2-oxoglutarate decarboxylase (menD)	9-31, 36-45, 59-67, 71-81, 86-94, 96-107, 111-122, 127-140, 153-168, 180-211, 218-224, 226-251, 256-270, 272-289, 299-305, 310-323, 334-341, 345-353, 358-364, 369-379, 384-390, 396-410, 417-423, 429-442, 454-464, 470-477, 497-505, 540-554	B:5; C:1	aa 400-413	B:SELAF54(404-413): 11/12	248, 277
ORF1326	autolysin AtlE (lytD)	6-25, 40-46, 75-81, 150-155, 200-205, 237-243, 288-295, 297-306, 308-320, 341-347, 356-363, 384-391, 417-429, 440-452, 465-473, 481-514, 540-546, 554-560, 565-577, 585-590, 602-609, 611-617, 625-634, 636-643, 661-668, 676-684, 718-724, 734-742, 747-754, 766-773, 775-781, 785-798, 800-807, 825-832, 840-857, 859-879, 886-892, 917-923, 950-956, 972-978, 987-1002, 1028-1035, 1049-1065, 1071-1099, 1111-1124, 1150-1172, 1185-1190, 1196-1207, 1234-1241, 1261-1271, 1276-1281, 1311-1320, 1325-1332	B:7; C:5	aa 1282-1298	B:SELAD20(1282-1298): 10/12	249, 278
ORF1333	quinol oxidase polypeptide iv (ec 1.9.3.-) (quinol oxidase aa3-600, subunit qoxd)	4-27, 33-55, 66-88	D:4	aa 3-93	D: nd	526, 575
ORF1356	hypothetical protein	9-36, 44-67, 74-97, 99-149, 161-181, 189-198, 211-224, 245-253, 267-273, 285-290, 303-324, 342-394, 396-427	D:32	aa54-95	D: nd	527, 597
ORF1373	dihydrolipoamide acetyltransferase	33-39, 42-78, 103-109, 126-136, 184-191, 225-232, 258-279, 287-294, 306-315, 329-334, 362-379, 381-404, 425-430	A:3, B:1	aa 124-188	A:SEFAP57(124-188): 2/12	250, 279
ORF1381	hypothetical protein	21-45, 62-67, 74-106, 108-142, 154-160, 230-236, 245-251, 298-305	D:5	aa7-44	D: nd	528, 576

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF1420	Muts2 protein, putative	8-32, 34-41, 46-55, 70-76, 81-89, 97-115, 140-148, 153-159, 165-171, 175-188, 207-239, 256-276, 280-289, 297-319, 321-335, 341-347, 352-360, 364-371, 384-411, 420-440, 449-460, 495-502, 505-516, 560-566, 573-588, 598-605, 607-614, 616-624, 674-694, 702-717	B:7	aa 581-608	B:SELAM40(581-604): 9/12	251, 280
ORF1443	cell division protein (divIB)	61-66, 111-117, 148-155, 173-182, 194-224, 263-293, 297-303, 313-321, 334-343, 345-356, 375-381, 384-395, 408-429, 448-454	D:4	aa175-229	D: nd	529, 577
ORF1500	Cell division protein FtsY	100-107, 154-167, 182-193, 200-206, 223-231, 233-243, 249-257, 265-273, 298-310, 326-336, 343-362, 370-384	A:2, B:3	aa 77-182	B:SELAP37(139-162): 9/12	252, 281
ORF1665	amino acid ABC transporter, permease protein	4-25, 44-55, 66-76, 82-90, 93-99, 104-109, 176-209, 227-242, 276-283, 287-328, 331-345, 347-376, 400-407, 409-416, 418-438, 441-474	D:5	aa 1-52	D: nd	530, 578
ORF1707	putative host cell surface-exposed lipoprotein	12-31, 40-69, 129-137, 140-151, 163-171, 195-202, 213-218	D:4	aa 20-76	D: nd	531, 598
ORF1786	D-3-phosphoglycerate dehydrogenase, putative	4-10, 16-32, 45-55, 66-78, 87-95, 103-115, 118-124, 135-150, 154-161, 166-174, 182-193, 197-207, 225-231, 252-261, 266-304, 310-315, 339-347, 351-359, 387-402, 411-423, 429-436, 439-450, 454-464, 498-505, 508-515	D:5	aa400-442	D: nd	532, 579
ORF1849	yhjN protein	8-51, 53-69, 73-79, 85-132, 139-146, 148-167, 179-205, 212-224, 231-257, 264-293, 298-304, 309-317, 322-351	D:5	aa254-301	D: nd	533, 580

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF1877	protein-export membrane protein SecD (secD-1)	6-19, 26-39, 41-51, 59-67, 72-85, 91-98, 104-111, 120-126, 147-153, 158-164, 171-178, 199-209, 211-218, 233-249, 251-257, 269-329, 362-368, 370-385, 392-420, 424-432, 454-489, 506-523, 534-539, 550-556, 563-573, 576-596, 603-642, 644-651, 655-666, 685-704, 706-733, 747-753	D:7	aa367-409	D: nd	534, 581
ORF1912	unknown con-served protein (conserved)	23-35, 37-70, 75-84, 90-112, 129-135, 137-151, 155-180, 183-209, 211-217, 219-225, 230-248, 250-269, 274-284, 289-320, 325-353, 357-371, 374-380, 384-399, 401-411,	D:4	aa131-187	D: nd	535, 582
ORF2015	Trehalose-6-phosphate hydrolase	8-17, 30-54, 82-89, 94-103, 157-166, 178-183, 196-204, 212-219, 222-227, 282-289, 297-307, 345-364, 380-393, 399-405, 434-439, 443-449, 453-475, 486-492, 498-507, 512-535, 538-548	A:3, B:8	aa 465-498	B:SELAH62(465-498): 5/12	253, 282
ORF2018	Glucose-6-phosphate 1-DH	4-16, 21-27, 39-51, 60-69, 76-83, 97-118, 126-132, 159-167, 171-177, 192-204, 226-240, 247-259, 281-286, 294-305, 314-320, 330-338, 353-361, 367-372, 382-392, 401-413, 427-434, 441-447, 457-463	B:17	aa 250-287	B:SELAI19(250-279): 3/12	254, 283
ORF2040	LysM domain protein protein	51-56, 98-108, 128-135, 138-144, 152-158, 177-192, 217-222, 232-251, 283-305, 406-431, 433-439	D:23	aa259-331	D: nd	536, 583
ORF2098	PilB related protein	13-18, 36-43, 45-50, 73-79, 95-100, 111-126, 133-139	A:60	aa 1-57	A:SEFAQ50(15-57): 5/12	255, 284
ORF2139	sodium:sulfate symporter family protein, putative	7-12, 22-97, 105-112, 121-128, 130-146, 152-164, 169-189, 192-203, 211-230, 238-246, 260-281, 304-309, 313-325, 327-357, 367-386, 398-444, 447-476, 491-512	D:41	aa42-118	D: nd	537, 584

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2172	SceB precursor (lytE)	4-23, 28-34, 38-43, 45-51, 63-71, 85-96, 98-112, 118-126, 167-174, 179-185, 219-228, 234-239, 256-263	A:438, B:40, D:4	aa 6-215	B:SELAH53(188-209): 3/12	256, 285
ORF2200	zinc ABC transporter, permease protein, putative	4-31, 33-40, 48-64, 66-82, 92-114, 118-133, 137-159, 173-246, 248-266	D:19	aa162-225	D: nd	538, 585
ORF2248	membrane protein, MmpL family, putative	4-11, 17-34, 72-78, 127-137, 178-227, 229-255, 262-334, 352-380, 397-405, 413-419, 447-454, 462-467, 478-490, 503-509, 517-558, 560-568, 571-576, 582-609, 623-629, 631-654, 659-710, 741-746, 762-767, 771-777, 788-793, 856-867	D:17	aa1-59, aa159-225, aa634-674	D: nd	539, 586
ORF2260	Unknown conserved protein in others	5-10, 18-29, 31-37, 66-178, 196-204, 206-213	B:4	aa 123-142	B:SELAG77(123-142): 12/12	257, 286
ORF2282	conserved hypothetical protein	16-22, 41-50, 52-64, 66-74, 89-95, 107-114, 123-130, 135-159, 167-181, 193-199, 223-231, 249-264, 279-289	A:4	aa 51-97	A:SEFAR88(51-97): 3/12	258, 287
ORF2376	DivIC homolog, putative	27-56, 102-107, 111-116	D:7	aa15-58	D: nd	540, 587
ORF2439	membrane-bound lytic murein transglycosidase D, putative	4-9, 11-26, 36-56, 59-73, 83-100, 116-130, 148-163, 179-193, 264-270, 277-287, 311-321	A:459, B:2, D:53	aa 10-217	B:SELAC31(75-129): 12/12	259, 288
ORF2493	conserved hypothetical protein	4-29, 37-77, 80-119	D:6	aa69-113	D: nd	541, 588
ORF2535	ATP-binding cassette transporter-like protein, putative	5-28, 71-81, 101-107, 128-135, 146-52, 178-188, 209-214, 224-233, 279-294, 300-306, 318-325, 342-347, 351-357	D:8	aa1-65	D: nd	542, 589

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2627	cation-transporting ATPase, E1-E2 family, putative	8-31, 34-80, 125-132, 143-153, 159-165, 176-189, 193-198, 200-206, 215-242, 244-262, 264-273, 281-289, 292-304, 318-325, 327-338, 347-371, 404-416, 422-429, 432-450, 480-488, 503-508, 517-525, 539-544, 551-562, 574-587, 600-631, 645-670	D:3	aa61-105	D: nd	543, 590
ORF2635	Hypothetical protein	4-10, 17-24, 26-42, 61-71, 90-96, 102-111, 117-125, 158-164, 173-182, 193-201, 241-255, 268-283, 289-298, 305-319, 340-353, 360-376, 384-390, 394-406	A:2, B:2	aa 139-169	B:SELAB63(138-163): 7/12	260, 289
ORF2669	Hypothetical protein	4-21, 35-42, 85-90, 99-105, 120-125, 148-155, 175-185, 190-196, 205-210, 217-225	A:14, B:8	aa 22-81	B:SELAE27(22-51): 5/12	261, 290
ORF2671	Hypothetical protein	4-23, 43-49, 73-84, 93-98, 107-113, 156-163, 179-190, 197-204, 208-218, 225-231, 248-255	A:44, B:14	aa 23-68	B:SELAD21(36-61): 5/12	262, 291
ORF2673	Hypothetical protein	4-20, 65-71, 99-105, 148-155, 171-182, 190-196, 204-210, 221-228, 240-246	A:16, B:3	aa 23-68	B:SELAE25(23-54): 2/12	263, 292
ORF2694	Hypothetical protein	4-26, 93-98, 121-132, 156-163, 179-192, 198-204, 212-220, 225-238	A:19, B:30	aa 25-82	B:SELAB26(27-60): 5/12	264, 293
ORF2695	Hypothetical protein	4-26, 43-50, 93-98, 107-113, 156-163, 179-190, 198-204, 212-218, 225-231, 247-254	A:7	aa 22-78	A:SEFAH77(22-66): 6/12	265, 294
ORF2719	two-component sensor histidine kinase, putative	5-52, 60-71, 75-84, 91-109, 127-135, 141-156, 163-177, 185-193, 201-214, 222-243, 256-262, 270-279, 287-293, 298-303, 321-328, 334-384, 390-404, 411-418, 427-435, 438-448, 453-479, 481-498, 503-509	B:4	aa 123-132	B:SELAA62(123-132): 6/12	266, 295
ORF2728	Accumulation-associated protein	4-13, 36-44, 76-86, 122-141, 164-172, 204-214, 235-242, 250-269, 291-299, 331-337, 362-369, 377-396, 419-427, 459-469, 505-524, 547-555, 587-597, 618-625, 633-652, 675-683, 715-727, 740-753, 761-780, 803-811, 842-853, 962-968, 1006-1020	A:265, B:448; C:4, D:9	aa 803-1001	B:SELAA10(850-878): 11/12	267, 296

S. epidermidis antigenic protein	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immuno-genic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2740	lipase precursor	4-21, 190-200, 218-228, 233-241, 243-261, 276-297, 303-312, 316-325, 346-352, 381-387, 436-442, 457-462, 495-505, 518-532, 543-557, 574-593	C:3	aa 110-177	C:GSBBL80(110-177):1/1	358, 364
ORF2764	oligopeptide ABC transporter, per-mease protein, putative	14-36, 62-131, 137-147, 149-162, 164-174, 181-207, 212-222, 248-268, 279-285	D:4	aa 6-41	D: nd	544, 591
ORF2767	unknown con-served protein in others	7-20, 22-35, 40-50, 52-61, 63-92, 94-101, 103-126, 129-155, 161-178, 192-198, 200-208, 210-229, 232-241, 246-273, 279-332, 338-359, 369-383	D:4	aa276-316	D: nd	545, 592
ORF2809	sodium:sulfate symporter family protein	4-29, 37-53, 56-82, 87-100, 108-117, 121-138, 150-160, 175-180, 189-195, 202-214, 220-247, 269-315, 324-337, 341-355, 361-412, 414-423, 425-440, 447-467	D:9	aa266-317, aa357-401	D: nd	546, 593
ORF2851	putative trans-membrane efflux protein	7-13, 20-32, 37-90, 93-103, 107-126, 129-155, 159-173, 178-189, 195-221, 234-247, 249-255, 268-303, 308-379	D:11	aa137-185	D: nd	547, 594

Table 2d: Immunogenic proteins identified by bacterial surface and ribosome display: S. aureus (new annotation)

Bacterial surface display: A, LSA250/1 library in fhuA with patient sera 1 (655); B, LSA50/6 library in lamB with patient sera 1 (484); C, LSA250/1 library in fhuA with IC sera 1 (571); E, LSA50/6 library in lamB with IC sera 2 (454); F, LSA50/6 library in lamB with patient sera P1 (1105); G, LSA50/6 library in lamb with IC sera 1 (471). Ribosome display: D, LSA250/1 library with IC sera (1686). **, prediction of antigenic sequences longer than 5 amino acids was performed with the programme ANTIGENIC (Kolaskar and Tongaonkar, 1990); #, identical sequence present twice in ORF.

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
SaA0003	ORF2967 & ORF2963	repC	7–19, 46–57, 85–91, 110–117, 125–133, 140–149, 156–163, 198–204, 236–251, 269–275, 283–290, 318–323, 347–363	B:3, C:14; F:29	aa 9–42 aa 156–241 aa 300–314 aa 343–420	C:GSBYI53(9–42):1/1 C:GSBYG39(156–241):1/1 C:GSBYM94(343–420):26/30	394, 396
ORF0123	ORF1909 – 18 aa at N–terminus	unknown	4–10, 25–30, 38–57, 91–108, 110–123, 125–144, 146–177, 179–198, 216–224, 226–233	B:3, E:7, G:1	aa 145–163	B:GSBXF80(150–163):5/27 E:GSBZC17(150–163):25/41	409, 410
ORF0160	ORF1941 –16 aa at N–terminus	unknown	4–26, 34–70, 72–82, 86–155, 160–166, 173–205, 207–228, 230–252, 260–268 , 280–313	A:1	aa 96–172	A:GSBXO07(96–172):5/30	411, 412
ORF0657	ORF unknown	LPXTGVI protein	9–33, 56–62, 75–84, 99–105, 122–127, 163–180, 186–192, 206–228, 233–240, 254–262, 275–283, 289–296, 322–330, 348–355, 416–424, 426–438, 441–452, 484–491, 541–549, 563–569, 578–584, 624–641	A:2, B:27, F:15	aa 526–544	B:GSBXE07–bdb1(527–542):11/71 F:SALAX70(526–544):11/41	413, 414
ORF1050	ORF1307 –4 aa at N–terminus	unknown	45–68, 72–79, 91–101, 131–142, 144–160, 179–201	A:1, H:45	aa 53–124	A:GSBXM26(53–124):7/30	415, 416
ORF1344	ORF0212 –10 aa at N–terminus	NifS protein homolog	13–26, 40–49, 61–68, 92–112, 114–123, 138–152, 154–183, 194–200, 207–225, 229–240, 259–265, 271–284, 289–309, 319–324, 330–336, 346–352, 363–372	A:11	aa 24–84	A:GSBXK59–bmd21(24–84):6/29	417, 418

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF1632	ORF1163 –4 aa at N-terminus	SdrH homolog	4-31, 50-55, 243-257, 259-268, 298-316, 326-335, 364-370, 378-407	B:6, E:11, F:34	aa 101-115 aa 115-139 aa 158-186	B:GSBXG53(164-182):39/71 F:SALAP07(101-115):11/41	419, 420
ORF2180	ORF0594 – 2 aa at N-terminus	LPXTGIV protein	9-17, 24-45, 67-73, 82-90, 100-107, 117-134, 137-145, 158-168, 176-183, 188-194, 206-213, 223-231, 243-248, 263-270, 275-282, 298-304, 344-355, 371-377, 382-388, 427-433, 469-479, 500-505, 534-559, 597-607, 662-687, 790-815, 918-943, 1032-1037, 1046-1060, 1104-1112, 1128-1137, 1179-1184, 1197-1204, 1209-1214, 1221-1239	A:3, C:3, E:6, F:2, H:6	aa 491-587 aa 633-715 aa 702-757# aa 758-830 (aa 830-885)#	A:GSBXS61(491-555):1/1 A:GSBXL64(494-585):1/1 A:GSBXS92(758-841):1/1 A:bmd4(702-757):16/30# (A:bmd4(830-885):16/30)# F:SALBC43(519-533):4/41	421, 422
ORF2184	ORF0590 – 8 aa at N-terminus	FnbpB	10-29, 96-116, 131-137, 146-158, 167-173, 177-182, 185-191, 195-201, 227-236, 260-266, 270-284, 291-299, 301-312, 348-356, 367-376, 382-396, 422-432, 442-453, 480-487, 497-503, 519-527, 543-548, 559-565, 579-585, 591-601, 616-623, 643-648, 657-663, 706-718, 746-758, 791-796, 810-817, 819-825, 833-839, 847-853, 868-885, 887-895, 919-932	A:2, C:4, G:9	aa 694-769 aa 774-847	A:GSBXM62(694-769):28/28 A:GSBXR22(774-847):1/1	423, 424
ORF2470	ORF0299 – 14 aa at N-terminus	Conserved hypothetical protein	4-27, 36-42, 49-55, 68-73, 94-101, 131-137, 193-200, 230-235, 270-276, 294-302, 309-324, 334-344, 347-364, 396-405, 431-437, 498-508, 513-519, 526-532, 539-544, 547-561, 587-594, 618-630, 642-653, 687-699, 713-719, 752-766	C:3	aa 400-441	C:GSBYH60(400-441):28/31	425, 426
ORF2498	ORF0267 ORF app. 580 aa longer at N terminus; plus other changes	Conserved hypothetical protein	8-19, 21-44, 63-76, 86-92, 281-286, 303-322, 327-338, 344-354, 364-373, 379-394, 405-412, 453-460, 501-506, 512-518, 526-542, 560-570, 577-583, 585-604, 622-630, 645-673, 677-691, 702-715, 727-741, 748-753, 770-785, 789-796, 851-858, 863-869, 876-881, 898-913, 917-924, 979-986, 991-997, 1004-1009, 1026-1041, 1045-1052, 1107-1114, 1119-1125, 1132-1137, 1154-1169, 1173-1192, 1198-1204, 1240-1254, 1267-1274, 1290-1298, 1612-1627	D:12, F:6	aa 358-411 aa 588-606 aa 895-909	D:17/21 F:SALAT38(895-909):8/41	427, 428

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF2548	ORF2711 -12 aa at N-terminus	IgG binding protein A	4-37, 44-53, 65-71, 75-82, 105-112, 126-132, 136-143, 164-170, 184-190, 194-201, 222-232, 242-248, 252-259, 280-291, 300-317, 413-420, 452-460, 485-503	A:55, B:54, C:35, F:59, G:56, H:38	aa 1-123 aa 207-273 aa 310-410	A:GSBXK68(1-73):21/30 A:GSBXK41(35-123):1/1 A:GSBXN38(207-273):19/30 A:GSBXL11(310-363):10/30 B:GSBXB22(394-406):37/71 F:SALAM17(394-406):29/41	429, 430
ORF2746	ORF2507 - 3 aa at N-terminus	homology with ORF1	4-9, 12-17, 40-46, 91-103, 106-113, 116-125, 150-160, 172-177, 182-188, 195-206, 241-261, 263-270, 277-285, 287-294	A:1, H:13	aa 63-126	A:GSBXO40(66-123):8/29	431, 432
ORF2797	ORF2470 -24 aa at N-terminus	unknown	13-32, 40-75, 82-95, 97-112, 115-121, 124-154, 166-192, 201-225, 227-252, 268-273, 288-297, 308-375, 379-434	B:3, E:2, F:13, H:3	aa 159-176 aa 325-339	B:GSBXE85(159-176):11/27 F:SALAQ47(159-176):8/41	433, 434
ORF2960	ORF2298 - 5 aa at N-terminus	putative Exotoxin	8-31, 35-44, 106-113, 129-135, 154-159, 168-178, 203-215, 227-236, 240-249, 257-266, 275-281, 290-296, 298-305, 314-319, 327-334	C:101, E:2, H:58	aa 1-80 aa 48-121 aa 98-190	C:GSBYG32(1-80)::6/7 C:GSBYG61-bhe2(48-116):26/30 C:GSBYN80(98-190):13/17	435, 436
ORF2963	ORF2295 -5 aa at N-terminus	putative Exotoxin	8-23, 35-41, 64-70, 81-87, 109-115, 121-132, 150-167, 177-188, 194-201, 208-216, 227-233, 238-248, 265-271, 279-285	C:3, E:3, G:1	aa 17-95	C:GSBYJ58(17-95):9/15	437, 438

S. aureus antigenic protein	Old ORF number	Putative function (by homology)	predicted immunogenic aa**	No. of selected clones per ORF and screen	Location of identified immunogenic region	Serum reactivity with relevant region (positive/total)	Seq ID no: (DNA +Prot)
ORF3200	ORF1331 +8506 aa at N-terminus	putative extracellular matrix binding protein	8-32, 45-52, 92-103, 154-159, 162-168, 207-214, 232-248, 274-280, 297-303, 343-349, 362-375, 425-442, 477-487, 493-498, 505-512, 522-533, 543-550, 558-564, 568-574, 580-600, 618-630, 647-652, 658-672, 692-705, 711-727, 765-771, 788-798, 812-836, 847-858, 870-898, 903-910, 1005-1015, 1018-1025, 1028-1036, 1058-1069, 1075-1080, 1095-1109, 1111-1117, 1119-1133, 1166-1172, 1183-1194, 1200-1205, 1215-1222, 1248-1254, 1274-1280, 1307-1317, 1334-1340, 1381-1391, 1414-1420, 1429-1439, 1445-1467, 1478-1495, 1499-1505, 1519-1528, 1538-1550, 1557-1562, 1572-1583, 1593-1599, 1654-1662, 1668-1692, 1701-1707, 1718-1724, 1738-1746, 1757-1783, 1786-1793, 1806-1812, 1815-1829, 1838-1848, 1853-1860, 1875-1881, 1887-1893, 1899-1908, 1933-1940, 1952-1961, 1964-1970, 1977-1983, 1990-1996, 2011-2018, 2025-2038, 2086-2101, 2103-2117, 2177-2191, 2195-2213, 2220-2225, 4"2237-2249, 2273-2279, 2298-2305, 2319-2327, 2349-2354, 2375-2381, 2391-2398, 2426-2433, 2436-2444, 2449-2454, 2463-2469, 2493-2499, 2574-2589, 2593-2599, 2605-2611, 2615-2624, 2670-2684, 2687-2698, 2720-2727, 2734-2754, 2762-2774, 2846-2866, 2903-2923, 2950-2956, 2985-2998, 3011-3031, 3057-3064, 2"3102-3117, 3137-3143, 3186-3195, 3211-3219, 3255-3270, 3290-3300, 3327-3334, 3337-3343, 3390-3396, 3412-3419, 3439-3446, 3465-3470, 3492-3500, 3504-3510, 3565-3573, 3642-3650, 3691-3698, 3766-3775, 3777-3788, 3822-3828, 3837-3847, 3859-3864, 3868-3879, 3895-3902, 3943-3951, 3963-3971, 3991-3997, 4018-4030, 4054-4060, 4074-4099, 4123-4129, 4147-4153, 4195-4201, 4250-4255, 4262-4267, 4270-4277, 4303-4310,	A:11, B:11, C:36, H:32	aa 8543-8601 aa 8461-8475	A:GSBXL07(8543-8601):6/28	439, 440

4321–4330, 4343–4352, 4396–4408, 4446–4451, 4471–4481, 4503–4509, 4516–4534, 4596–4604, 4638–4658, 4698–4710, 4719–4732, 4776–4783, 4825–4833, 4851–4862, 4882–4888, 4894–4909, 4937–4942, 5047–5054, 5094–5100, 5102–5112, 5120–5125, 5146–5153, 5155–5164, 5203–5214, 5226–5236, 5278–5284, 5315–5321, 5328–5342, 5348–5359, 5410–5420, 5454–5466, 5481–5489, 5522–5538, 5597–5602, 5607–5614, 0"5623–5629, 5650–5665, 5707–5719, 5734–5742, 5772–5778, 5785–5790, 5833–5845, 5857–5863, 5899–5904, 5908–5921, 5959–5971, 5981–5989, 6010–6017, 6034–6043, 6058–6064, 6112–6120, 6154–6169, 6210–6217, 6231–6240, 6261–6268, 6288–6294, 6318–6324, 6340–6349, 6358–6369, 6402–6407, 6433–6438, 6483–6493, 6513–6519, 6527–6546, 6561–6574, 6599–6608, 6610–6616, 6662–6673, 6696–6705, 6729–6743, 6769–6775, 6792–6801, 6819–6828, 6840–6846, 6860–6870, 6915–6928, 6966–6972, 7021–7028, 7032–7047, 7096–7101, 7109–7117, 7138–7149, 7157–7162, 7201–7206, 7238–7253, 7283–7294, 7296–7302, 7344–7365, 7367–7376, 7389–7404, 7413–7433, 7475–7482, 7493–7500, 7535–7549, 7596–7608, 7646–7651, 7661–7678, 7722–7731, 7741–7754, 7764–7769, 7776–7782, 7791–7806, 7825–7837, 7862–7875, 7891–7897, 7922–7931, 7974–7981, 7999–8005, 8039–8045, 8049–8065, 8070–8075, 8099–8112, 8119–8125, 8151–8158, 8169–8181, 8226–8232, 8258–8264, 8291–8299, 8301–8310, 8325–8335, 8375–8389, 8394–8400, 8405–8412, 8421–8436, 8478–8485, 8512–8521, 8528–8538, 8564–8579, 8587–8594, 8603–8615, 8626–8637, 8640–8646, 8657–8672, 8684–8691, 8725–8736, 8748–8761, 8777–8783, 8794–8799, 8810–8825, 8851–8862, 8874–8887, 8903–8912, 8914–8926, 8933–8943, 8954–8960, 8979–8988, 9004–9011, 9035–9041, 9056–9069, 9077–9086, 9088–9096, 9106–9111, 9124–9133, 9183–9191, 9224–9231, 9235–9241, 9250–9265, 9279–9290, 9295–

			9300, 9326–9343, 9408–9414, 9422–9427, 9435–9441, 9455–9461, 9507–9517, 9532–9538, 9580–9589, 9594–9600, 9614–9623, 9643–9648, 9665–9683, 9688–9700, 9720–9726, 9742–9758, 9767–9775, 9795–9800, 9817–9835, 9842–9847, 9912–9919, 9925–9938, 9943–9963, 9970–10009, 10025–10031, 10037–10043, 10045–10063, 10066–10073, 10117–10124, 10126–10136, 10203–10210, 10218–10225, 10232–10242, 10287–10292, 10303–10323, 10352–10360, 10385–10396, 10425–10431, 10452–10459, 10480–10485				

Table 3. Serological proteome analysis of S. aureus surface proteins using human sera

a) *S. aureus/agr* "stress conditions"

Spot ID/sera	IC40 1:20,000	IC35, N26, C4 1:50,000 each	Infant pool C2,5,6,10,12 1:10,000	N22 1:10.000 IC40 1:50,000
PCK2	+	+	–	+
PCK4	+	+++	–	+++
PCK5	–	(+)	–	+
PCK6	+	+	–	+

Spot ID/sera	IC35, 40 1:50,000 N22 1:10,000	P-pool (P6,18,25,28,29) 1:50,000 each	Infant pool C2,5,6,10,12 1:10,000	
PAC1	++	++	–	
PAC2	++	+++	–	
PAC3	–	+	–	
PAC5	–	++	–	

Spot ID/sera	P-pool (P6,18,25,28,29) 1:50,000 each	Infant 14 1:10,000	IC pool / IgG (N26, IC34,35) 1:30,000 each	IC pool / IgA (N26, IC34,35) 1:30,000 each
PAC11	++	–	++	++
PAC12	++	–	++	++
PAC13	–	–	–	++
PAC14	–	–	+	+
PAC15	–	–	+++	+++
PAC16	+	–	+	+
PAC17	+	–	+	+
PAC18	++	–	–	–
PAC19	–	–	++	++
PAC20	++	–	–	–
POV31	+++	–	–	–
POV32	+	–	–	–
POV33	+	–	–	–
POV34	+	–	–	–
POV35	+	–	–	–
P OV36	+	–	–	–
P OV37	++	–	–	–

P OV38	++	–	–	–
P OV39	+++	–	–	–
P OV40	+++	–	–	–

b) S. aureus/COL "standard conditions"

Spot ID/sera	IC pool (N26,IC34,35) 1:30,000 each	IC35 1:20,000	P18 1:10,000	P25 1:10,000	P1 1:5,000	P29 1:2,500	Infant 18 1:10,000
POV2	+++	+++	+++	+++	+++	–	–
POV3.1	+++	+++	+++	+++	+++	–	–
POV3.2	+++	+++	+++	+++	+++	–	–
POV4	+	+++	–	–	–	–	–
POV7	–	–	+++	–	–	–	–
POV10	–	++	(+)	(+)	–	(+)	–
POV12	–	–	–	–	–	+++	–
POV13	++	+++	+++	+++	++	++	–
POV14	++	+++	+++	++	++	++	–
POV15	+	+	–	+	(+)	–	–

c) S. aureus/COL "stress conditions"

Spot ID/sera	P-pool (P6,18,25,28,29) 1:50,000 each	IC34+IC35 1:20,000 each	P18 1:10,000	P29 1:10,000	Infant 14 1:10,000
POV16	–	+++	–	–	–
POV17	–	+++	(+)	–	–
POV18	+	–	++	–	–
POV19	(+)	–	+++	–	–
POV21	–	–	+	–	–
POV23	–	+	–	–	–
POV24	–	+	–	–	–
POV25	+	–	–	–	–

Table 4. S. aureus antigens identified by MALDI-TOF-MS sequencing (ORFs in bold were also identified by bacterial surface display)

Prediction of antigenic regions in selected antigens identified by serological proteome analysis using human sera

spot ID	S. aureus protein (ORF no. / abbrev.)	Putative function (by homology)	Seq ID no: (DNA, Prot)	Putative localization
PCK2	ORF0599	Glycinamide-ribosyl synthase	107, 108	cytoplasmic
PCK5	ORF0484 yitU	conserved hypoth. protein (yitU)	109, 110	cytoplasmic
PCK6	ORF2309 mqo	membrane-associated malate-quinone oxidase	111, 112	peripheral membrane
POV2	ORF0766 aux1	protein phosphatase contributing to methicilin resistance	113, 114	trans-membrane
POV4, 17 PAC14, 19	ORF0078 EF-Tu	C-terminal part of 44 kDa protein similar to elongation factor Tu	115, 116	cytoplasmic/ secreted
POV5 [1)]	ORF0782	3-ketoacyl-acyl carrier protein reductase (fabG)	117, 118	cytoplasmic
POV7	**ORF0317** SecA	protein transport across the membrane SecA	39, 91	cytoplasmic
POV10	ORF1252 yrzC	hypothetical BACSU 11.9 kd protein (upf0074 (rff2) family)	119, 120	cytoplasmic
POV12	ORF0621 pdhB	dihydrolipoamide acetyltransferase (pdhB)	121, 122	cytoplasmic
POV14	ORF0072 rpoB	DNA-directed RNA polymerase ß	125, 126	cytoplasmic
POV15	ORF0077 EF-G	85 kD vitronectin binding protein	127, 128	cytoplasmic
POV18	not found YLY1	general stress protein YLY1	129, 130	cytoplasmic
POV30 [1)]	ORF0069 RL7	ribosomal protein L7	131, 132	cytoplasmic
POV21	ORF0103 yckG	probable hexulose-6-phosphate synthase (yckG)	133, 134	cytoplasmic
,POV24	ORF0419 yurX	conserved hypothetical protein (yurX)	137, 138	cytoplasmic

spot ID	S. aureus pro-tein (ORF no. / ab-brev.)	Putative function (by homology)	Seq ID no: (DNA, Prot)	Putative local-ization
POV25	ORF2441 gidA	glucose inhibited division protein a (gidA)	139, 140	cytoplasmic
PAC1	ORF1490 prsA	protein export protein prsa precursor (prsA)	173, 174	periplasmic
PAC2	ORF1931 ModA	periplasmic molybdate binding protein (ModA)	175, 176	surface
PAC3	ORF2053	heavy metal dependent transcriptional activator, putative regulator of multidrug resistance efflux pump pmrA	177, 178	cytoplasmic
PAC5	ORF2233 ydaP	pyruvate oxidase (ydaP)	179, 180	cytoplasmic
PAC11	ORF1361	LPXTGV, extracellularmatrix-bdg.	3, 56	surface
PAC12	ORF1244 alaS	alanyl-tRNA synthetase	159, 160	cytoplasmic
PAC13	ORF0835 ymfA	RNA processing enzyme/ATP-bdg.	161, 162	cytoplasmic
PAC15	ORF1124 bfmBB	lipoamid acyltransferase component of branched-chain alpha-keto acid dehy-drogenase complex	163, 164	cytoplasmic
PAC16	ORF0340 GAPDH	glyceraldehydes-3-phosphate dehydrogenase	165, 166	cytoplasmic
PAC17	not found Contig83	5'-methylthioadenosine nucleosidase / S-adenosylhomo-cysteine nucleosidase		cytoplasmic
PAC20	ORF2711	75% identity to ORF2715 similar to hypothetical proteins	167, 168	unknown
POV31	ORF0659	29 kDa surface protein	236, 238	surface
POV32	ORF0659	29 kDa surface protein	236, 238	surface
POV33	ORF0659	29 kDa surface protein	236, 238	surface
POV34	ORF0659	29 kDa surface protein	236, 238	surface
POV35	ORF0659	29 kDa surface protein	236, 238	surface
P OV36	ORF00661	LPXTG-motif cell wall anchor domain protein	235, 237	surface
P OV37	ORF0659	29 kDa surface protein	236, 238	surface

spot ID	S. aureus protein (ORF no. / abbrev.)	Putative function (by homology)	Seq ID no: (DNA, Prot)	Putative localization
P OV38	ORF0659	29 kDa surface protein	236, 238	surface
P OV39	ORF0657	LPXTG-anchored surface protein	1, 142	surface
P OV40	not identified			

Seq ID no: (Protein)	spot ID	S. aureus ORF no. / abbrev.	Putative localization	Putative antigenic surface areas (Antigenic package)
112	PCK6	ORF2309 mqo	peripheral membrane	61-75, 82-87, 97-104, 113-123, 128-133, 203-216, 224-229, 236-246, 251-258, 271-286, 288-294, 301-310, 316-329, 337-346, 348-371, 394-406, 418-435, 440-452
114	POV2	ORF766 aux1	trans-membrane	30-37, 44-55, 83-91, 101-118, 121-128, 136-149, 175-183, 185-193, 206-212, 222-229, 235-242
116	POV4	ORF078 EF-Tu	cytoplasmic/ secreted	28-38, 76-91, 102-109, 118-141, 146-153, 155-161, 165-179, 186-202, 215-221, 234-249, 262-269, 276-282, 289-302, 306-314, 321-326, 338-345, 360-369, 385-391
176	PAC2	ORF1931 ModA	periplasmic	29-44, 74-83, 105-113, 119-125, 130-148, 155-175, 182-190, 198-211, 238-245
174	PAC1	ORF1490 prsA	periplasmic	5-24, 38-44, 100-106, 118-130, 144-154, 204-210, 218-223, 228-243, 257-264, 266-286, 292-299
168	PAC20	ORF2711	unknown	7-14, 21-30, 34-50, 52-63, 65-72, 77-84, 109-124, 129-152, 158-163, 175-190, 193-216, 219-234

spot ID	GI no. or TIGR no.	S. aureus protein (ORF no. / abbrev.)	Putative function (by homology)	Seq ID no: (DNA, Prot)
PCK2	TIGR1280	ORF0599	Glycinamide-ribosyl synthase	107, 108

PCK4	7672993	ORF2268 IsaA	possibly adhesion/aggregation	12, 64
PCK5	TIGR6209	ORF0484 yitU	conserved hypoth. protein (yitU)	109, 110
PCK6	TIGR6182	ORF2309	membrane-associated malate-quinone oxidase	111, 112
POV2	6434044	ORF0766 aux1	protein phosphatase contributing to methi-cilin resistance	113, 114
POV3.1	7672993	ORF2268 IsaA	possibly adhesion/aggregation	12, 64
POV3.2	7672993	ORF2268 IsaA	possibly adhesion/aggregation	12, 64
POV4	TIGR8079	ORF0078 EF-Tu	C-terminal part of 44 kDa protein similar to elongation factor Tu	115, 116
POV5 [1)]	TIGR8091	ORF0782	3-ketoacyl-acyl carrier protein reductase (fabG)	117, 118
POV7	2500720	ORF0317 SecA	protein transport across the membrane SecA	39, 91
POV10	TIGR8097	ORF1252 yrzC	hypothetical BACSU 11.9 kd protein (upf0074 (rff2) family)	119, 120
POV12	2499415	ORF0621 pdhB	dihydrolipoamide acetyltransferase (pdhB)	121, 122
POV13	7470965	ORF0094 SdrD	fibrinogen-bdg. (LPXTG) protein homolog (SdrD)	123, 124
POV14	1350849	ORF0072 rpoB	DNA-directed RNA polymerase β	125, 126
POV15	6920067	ORF0077 EF-G	85 kD vitronectin binding protein	127, 128
POV17	TIGR8079	ORF0078	C-terminal part of 44 kDa protein similar to elongation factor Tu	115, 116
POV18	3025223	not found	general stress protein YLY1	129, 130
POV30 [1)]	350771	ORF0069 RL7	ribosomal protein L7	131, 132
POV21		ORF0103	probable hexulose-6-phosphate synthase (yckG)	133, 134
POV23		ORF0182	lipoprotein (S.epidermis)	135, 136

[1)] identified from a total lysate from S. aureus 8325-4 spa- grown under standard conditions. Seroreactivity with 1/1 patient and 2/4 normal sera but not with infant serum (C5).

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C l a i m s :

1. Method for identification, isolation and production of hyperimmune serum-reactive antigens from a pathogen, a tumor, an allergen or a tissue or host prone to auto-immunity, said antigens being suited for use in a vaccine for a given type of animal or for humans, characterized by the following steps:

- providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity,
- providing at least one expression library of said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity
- screening said at least one expression library with said antibody preparation,
- identifying antigens which bind in said screening to antibodies in said antibody preparation,
- screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen, tumor, allergen or tissue or host prone to auto-immunity,
- identifying the hyperimmune serum-reactive antigen portion of said identified antigens and which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera and
- optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods.

2. Method for identification, isolation and production of a practically complete set of hyperimmune serum-reactive antigens of a specific pathogen, said antigens being suited for use in a vaccine for a given type of animal or for humans, characterized by the following steps:

- providing an antibody preparation from a plasma pool of said given type of animal or from a human plasma pool or individual sera with antibodies against said specific pathogen,
- providing at least three different expression libraries of said specific pathogen,

•screening said at least three different expression libraries with said antibody preparation,
•identifying antigens which bind in at least one of said at least three screenings to antibodies in said antibody preparation,
•screening the identified antigens with individual antibody preparations from individual sera from individuals with antibodies against said specific pathogen,
•identifying the hyperimmune serum-reactive antigen portion of said identified antigens which hyperimmune serum-reactive antigens bind to a relevant portion of said individual antibody preparations from said individual sera,
•repeating said screening and identification steps at least once,
•comparing the hyperimmune serum-reactive antigens identified in the repeated screening and identification steps with the hyperimmune serum-reactive antigens identified in the initial screening and identification steps,
•further repeating said screening and identification steps, if at least 5% of the hyperimmune serum-reactive antigens have been identified in the repeated screening and identification steps only, until less than 5 % of the hyperimmune serum-reactive antigens are identified in a further repeating step only to obtain a complete set of hyperimmune serum-reactive antigens of a specific pathogen and
•optionally isolating said hyperimmune serum-reactive antigens and producing said hyperimmune serum-reactive antigens by chemical or recombinant methods.

3. Method according to claim 1 or 2 characterized in that at least one of said expression libraries is selected from a ribosomal display library, a bacterial surface library and a proteome.

4. Method according to claim 2 characterized in that said at least three different expression libraries are at least a ribosomal display library, a bacterial surface library and a proteome.

5. Method according to any one of claims 1 to 4, characterized

in that said plasma pool is a human plasma pool taken from individuals having experienced or are experiencing an infection with said pathogen.

6. Method according to any one of claims 1 to 5, characterized in that said expression libraries are genomic expression libraries of said pathogen.

7. Method according to any one of claims 1 to 6, characterized in that said expression libraries are complete genomic expression libraries, preferably with a redundancy of at least 2x, more preferred at least 5x, especially at least 10x.

8. Method according to any one of claims 1 to 7, characterized in that it comprises the steps of screening at least a ribosomal display library, a bacterial surface display library and a proteome with said antibody preparation and identifying antigens which bind in at least two, preferably which bind to all, of said screenings to antibodies in said antibody preparation.

9. Method according to any one of claims 1 to 8, characterized in that said pathogen is selected from the group of bacterial, viral, fungal and protozoan pathogens.

10. Method according to any one of claims 1 to 9, characterized in that said pathogen is selected from the group of human immunedeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, Rous sarcoma virus, Epstein-Barr virus, influenza virus, rotavirus, Staphylococcus aureus, Staphylococcus epidermidis, Chlamydia pneumoniae, Chlamydia trachomatis, Mycobacterium tuberculosis, Mycobacterium leprae, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis, Bacillus anthracis, Vibrio cholerae, Borrelia burgdorferi, Plasmodium sp., Aspergillus sp. or Candida albicans.

11. Method according to any one of claims 1 to 10, characterized in that at least one of said expression libraries is a ribosomal display library or a bacterial surface display library and said hyperimmune serum-reactive antigens are produced by expression of the coding sequences of said hyperimmune serum-reactive antigens

contained in said library.

12. Method according to any one of claims 1 to 11, characterized in that said produced hyperimmune serum-reactive antigens are finished to a pharmaceutical preparation, optionally by addition of a pharmaceutically acceptable carrier and/or excipient.

13. Method according to claim 12, characterized in that said pharmaceutical preparation is a vaccine.

14. Method according to claim 12 or 13, characterized in that said pharmaceutically acceptable carrier and/or excipient is an immunostimulatory compound.

15. Method according to claim 14, characterized in that said immunostimulatory compound is selected from the group of polycationic substances, especially polycationic peptides, immunostimulatory deoxynucleotides, alumn, Freund's complete adjuvans, Freund's incomplete adjuvans, neuroactive compounds, especially human growth hormone, or combinations thereof.

16. Method according to any one of claims 1 to 15, characterized in that said individual antibody preparations are derived from patients with acute infection with said pathogen, especially from patients with an antibody titer to said pathogen being higher than 80%, preferably higher than 90%, especially higher than 95% of human patient or carrier sera tested.

17. Method according to any one of claims 1 to 16, characterized in that at least 10, preferably at least 30, especially at least 50, individual antibody preparations are used in identifying said hyperimmune serum-reactive antigens.

18. Method according to any one of said claims 1 to 17, characterized in that said relevant portion of said individual antibody preparations from said individual sera are at least 10, preferably at least 30, especially at least 50 individual antibody preparations, and/or at least 20 %, preferably at least 30 %, especially at least 40 %, of all individual antibody preparations used in said screening.

19. Method according to any one of claims 1 to 18, characterized in that said individual sera are selected by having an IgA titer against a lysate, cell wall components or recombinant proteins of said pathogen being above 4000 U, especially above 6000 U, and/or by having an IgG titer being above 10000 U, preferably above 12000 U.

20. Method according to any one of claims 1 to 19, characterized in that said pathogen is a Staphylococcus pathogen, especially Staphylococcus aureus. and/or Staphylococcus epidermidis.

21. A hyperimmune serum-reactive antigen selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3, 4 and 5, especially selected from the group consisting of Seq.ID No. 56, 57, 59, 60, 67, 70, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 85, 87, 88, 89, 90, 92, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 126, 128, 130, 132, 134, 138, 140, 142, 151, 152, 154, 155 and hyperimmune fragments thereof.

22. A hyperimmune serum-reactive antigen obtainable by a method according to any one of claims 1 to 20 and being selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3, 4 and 5, especially selected from the group consisting of Seq.ID No. 56, 57, 59, 60, 67, 70, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 85, 87, 88, 89, 90, 92, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 126, 128, 130, 132, 134, 138, 140, 142, 151, 152, 154, 155 and hyperimmune fragments thereof.

23. Use of a hyperimmune serum-reactive antigen selected from the group consisting of the sequences listed in any one of Tables 2a, 2b, 2c, 2d, 3, 4 and 5, especially selected from the group consisting of Seq.ID No. 55, 56, 57, 58, 59, 60, 62, 66, 67, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 92, 94, 95, 96, 97, 99, 100, 101, 102, 103, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 126, 128, 130, 132, 134, 138, 140, 142, 151, 152, 154, 155, 158 and hyperimmune fragments thereof for the manufacture of a pharmaceutical preparation, es-

pecially for the manufacture of a vaccine against staphylococcal infections or colonization in particular against Staphylococcus aureus or Staphylococcus epidermidis.

24. Hyperimmune fragment of a hyperimmune serum-reactive antigen selected from the group consisting of peptides comprising the amino acid sequences of column "predicted immunogenic aa", "Location of identified immunogenic region" and "Serum reactivity with relevant region" of Tables 2a, 2b, 2c and 2d and the amino acid sequences of column "Putative antigenic surface areas" of Table 4 and 5, especially peptides comprising amino acid No. aa 12-29, 34-40, 63-71, 101-110, 114-122, 130-138, 140-195, 197-209, 215-229, 239-253, 255-274 and 39-94 of Seq.ID No. 55,
aa 5-39, 111-117, 125-132, 134-141, 167-191, 196-202, 214-232, 236-241, 244-249, 292-297, 319-328, 336-341, 365-380, 385-391, 407-416, 420-429, 435-441, 452-461, 477-488, 491-498, 518-532, 545-556, 569-576, 581-587, 595-602, 604-609, 617-640, 643-651, 702-715, 723-731, 786-793, 805-811, 826-839, 874-889, 37-49, 63-77 and 274-334, of Seq.ID No.56,
aa 28-55, 82-100, 105-111, 125-131, 137-143, 1-49, of Seq.ID No. 57,
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aa 16-38, 71-77, 87-94, 105-112, 124-144, 158-164, 169-177, 180-186, 194-204, 221-228, 236-245, 250-267, 336-343, 363-378, 385-394, 406-412, 423-440, 443-449, 401-494, of Seq.ID No. 59,
aa 18-23, 42-55, 69-77, 85-98, 129-136, 182-188, 214-220, 229-235, 242-248, 251-258, 281-292, 309-316, 333-343, 348-354, 361-367, 393-407, 441-447, 481-488, 493-505, 510-515, 517-527, 530-535, 540-549, 564-583, 593-599, 608-621, 636-645, 656-670, 674-687, 697-708, 726-734, 755-760, 765-772, 785-792, 798-815, 819-824, 826-838, 846-852, 889-904, 907-913, 932-939, 956-964, 982-1000, 1008-1015, 1017-1024, 1028-1034, 1059-1065, 1078-1084, 1122-1129, 1134-1143, 1180-1186, 1188-1194, 1205-1215, 1224-1230, 1276-1283, 1333-1339, 1377-1382, 1415-1421, 1448-1459, 1467-1472, 1537-1545, 1556-1566, 1647-1654, 1666-1675, 1683-1689, 1722-1737, 1740-1754, 1756-1762, 1764-1773, 1775-1783, 1800-1809, 1811-1819, 1839-1851, 1859-1866, 1876-1882, 1930-1939, 1947-1954, 1978-1985, 1999-2007, 2015-2029, 2080-2086, 2094-2100, 2112-2118, 2196-2205,

2232-2243, 198-258, 646-727 and 2104-2206, of Seq.ID No. 60, aa 10-29, 46-56, 63-74, 83-105, 107-114, 138-145, 170-184, 186-193, 216-221, 242-248, 277-289, 303-311, 346-360, 379-389, 422-428, 446-453, 459-469, 479-489, 496-501, 83-156, of Seq.ID No. 62,
aa 14-22, 32-40, 52-58, 61-77, 81-93, 111-117, 124-138, 151-190, 193-214, 224-244, 253-277, 287-295, 307-324, 326-332, 348-355, 357-362, 384-394, 397-434, 437-460, 489-496, 503-510, 516-522, 528-539, 541-547, 552-558, 563-573, 589-595, 602-624, 626-632, 651-667, 673-689, 694-706, 712-739, 756-790, 403-462, of Seq.ID No. 66,
aa 49-56, 62-68, 83-89, 92-98, 109-115, 124-131, 142-159, 161-167, 169-175, 177-188, 196-224, 230-243, 246-252, 34-46, of Seq.ID No. 67,
aa 11-20, 26-47, 69-75, 84-92, 102-109, 119-136, 139-147, 160-170, 178-185, 190-196, 208-215, 225-233, 245-250, 265-272, 277-284, 300-306, 346-357, 373-379, 384-390, 429-435, 471-481, 502-507, 536-561, 663-688, 791-816, 905-910, 919-933, 977-985, 1001-1010, 1052-1057, 1070-1077, 1082-1087, 1094-1112, 493-587, 633-715 and 704-760, of Seq.ID No.70,
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aa 10-26, 31-43, 46-58, 61-66, 69-79, 85-92, 100-115, 120-126, 128-135, 149-155, 167-173, 178-187, 189-196, 202-222, 225-231, 233-240, 245-251, 257-263, 271-292, 314-322, 325-334, 339-345, 59-74, of Seq.ID No. 72,
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aa 5-22, 42-50, 74-81, 139-145, 167-178, 220-230, 246-253, 255-264, 137-237 and 250-267, of Seq.ID No. 74,
aa 10-26, 31-44, 60-66, 99-104, 146-153, 163-169, 197-205, 216-223, 226-238, 241-258, 271-280, 295-315, 346-351, 371-385, 396-407, 440-446, 452-457, 460-466, 492-510, 537-543, 546-551, 565-582, 590-595, 635-650, 672-678, 686-701, 705-712, 714-721, 725-731, 762-768, 800-805, 672-727, of Seq.ID No. 75,
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aa 7-35, 54-59, 247-261, 263-272, 302-320, 330-339, 368-374, 382-411, 126-143 and 168-186, of Seq.ID No. 77,
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278, 305-313, 321-327, 334-341, 31-61 and 58-74, of Seq.ID No. 78,
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aa 9-28, 43-48, 56-75, 109-126, 128-141, 143-162, 164-195, 197-216, 234-242, 244-251, 168-181, of Seq.ID No. 87,

aa 4-10, 20-42, 50-86, 88-98, 102-171, 176-182, 189-221, 223-244, 246-268, 276-284, 296-329, 112-188, of Seq.ID No. 88,
aa 4-9, 13-24, 26-34, 37-43, 45-51, 59-73, 90-96, 99-113, 160-173, 178-184, 218-228, 233-238, 255-262, 45-105, 103-166 and 66-153, of Seq.ID No. 89,
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aa 26-53, 95-123, 164-176, 189-199, 8-48, of Seq.ID No. 92,
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aa 13-36, 40-49, 111-118, 134-140, 159-164, 173-183, 208-220, 232-241, 245-254, 262-271, 280-286, 295-301, 303-310, 319-324, 332-339, 1-85, 54-121 and 103-185, of Seq.ID No. 95,
aa 39-44, 46-80, 92-98, 105-113, 118-123, 133-165, 176-208, 226-238, 240-255, 279-285, 298-330, 338-345, 350-357, 365-372, 397-402, 409-415, 465-473, 488-515, 517-535, 542-550, 554-590, 593-601, 603-620, 627-653, 660-665, 674-687, 698-718, 726-739, 386-402, of Seq.ID No. 96,
aa 5-32, 34-49, 1-43, of Seq.ID No. 97,
aa 10-27, 37-56, 64-99, 106-119, 121-136, 139-145, 148-178, 190-216, 225-249, 251-276, 292-297, 312-321, 332-399, 403-458, 183-200, of Seq.ID No. 99,
aa 5-12, 15-20, 43-49, 94-106, 110-116, 119-128, 153-163, 175-180, 185-191, 198-209, 244-252, 254-264, 266-273, 280-288, 290-297, 63-126, of Seq.ID No. 100,
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aa 12-36, 43-50, 58-65, 73-78, 80-87, 108-139, 147-153, 159-172, 190-203, 211-216, 224-232, 234-246, 256-261, 273-279, 286-293, 299-306, 340-346, 354-366, 167-181, of Seq.ID No. 106,
aa 61-75, 82-87, 97-104, 113-123, 128-133, 203-216, 224-229, 236-246, 251-258, 271-286, 288-294, 301-310, 316-329, 337-346,

348-371, 394-406, 418-435, 440-452 of Seq.ID No. 112,
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aa 9-33, 56-62,75-84, 99-105, 122-127, 163-180, 186-192, 206-228, 233-240, 254-262, 275-283, 289-296, 322-330, 348-355, 416-424, 426-438, 441-452, 484-491, 522-528, 541-549, 563-569, 578-584, 624-641, 527-544, of Seq.ID No. 142,
aa 37-42, 57-62, 121-135, 139-145, 183-190, 204-212, 220-227, 242-248, 278-288, 295-30, 304-309, 335-341, 396-404, 412-433, 443-449, 497-503, 505-513, 539-545, 552-558, 601-617, 629-649, 702-711, 736-745, 793-804, 814-829, 843-858, 864-885, 889-895, 905-913, 919-929, 937-943, 957-965, 970-986, 990-1030, 1038-1049, 1063-1072, 1080-1091, 1093-1116, 1126-1136, 1145-1157, 1163-1171, 1177-1183, 1189-1196, 1211-1218, 1225-1235, 1242-1256, 1261-1269, 624-684, of Seq.ID No. 151,
aa 8-23, 31-38, 42-49, 61-77, 83-90, 99-108, 110-119, 140-147, 149-155, 159-171, 180-185, 189-209, 228-234, 245-262, 264-275, 280-302, 304-330, 343-360, 391-409, 432-437, 454-463, 467-474, 478-485, 515-528, 532-539, 553-567, 569-581, 586-592, 605-612, 627-635, 639-656, 671-682, 700-714, 731-747, 754-770, 775-791, 797-834, 838-848, 872-891, 927-933, 935-942, 948-968, 976-986, 1000-1007, 1029-1037, 630-700, of Seq.ID No. 152,
aa 17-25, 27-55, 84-90, 95-101, 115-121, 55-101, of Seq.ID No. 154,
aa 13-28, 40-46, 69-75, 86-92, 114-120, 126-137, 155-172, 182-193, 199-206, 213-221, 232-238, 243-253, 270-276, 284-290, 22-100, of Seq.ID No. 155 and
aa 7-19, 46-57, 85-91, 110-117, 125-133, 140-149, 156-163, 198-204, 236-251, 269-275, 283-290, 318-323, 347-363, 9-42 and 158-174, of Seq.ID No. 158,
aa 7-14, 21-30, 34-50, 52-63, 65-72, 77-84, 109-124, 129-152, 158-163, 175-190, 193-216, 219-234 of Seq.ID.No. 168,
aa 5-24, 38-44, 100-106, 118-130, 144-154, 204-210, 218-223, 228-243, 257-264, 266-286, 292-299 of Seq.ID.No. 174,
aa 29-44, 74-83, 105-113, 119-125, 130-148, 155-175, 182-190, 198-211, 238-245 of Seq.ID.No. 176, and fragments as depicted in Tables 2 and 4 and fragments comprising at least 6, preferably

more than 8, especially more than 10 aa of said sequences.

25. Helper epitopes of an antigen or a fragment, as defined in anyone of claims 21 to 24, especially peptides comprising fragments selected from the peptides mentioned in column "Putative antigenic surface areas" in Table 4 and 5 and from the group aa 6-40, 583-598, 620-646 and 871-896 of Seq.ID.No.56, aa 24-53 of Seq.ID.No.70, aa 240-260 of Seq.ID.No.74, aa 1660-1682 and 1746-1790 of Seq.ID.No. 81, aa 1-29, 680-709, and 878-902 of Seq.ID.No. 83, aa 96-136 of Seq.ID.No. 89, aa 1-29, 226-269 and 275-326 of Seq.ID.No. 94, aa 23-47 and 107-156 of Seq.ID.No. 114 and aa 24-53 of Seq.ID.No. 142 and fragments thereof being T-cell epitopes.

26. Vaccine comprising a hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of claims 21 to 25.

27. Vaccine according to claim 25, characterized in that it further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, especially polycationic peptides, immunostimulatory deoxynucleotides (ODNs), neuroactive compounds, especially human growth hormone, alumn, Freund's complete or incomplete adjuvans or combinations thereof.

28. Preparation comprising antibodies against at least one antigen or a fragment thereof, as defined in any one of claims 21 to 25.

29. Preparation according to claim 27, characterized in that said antibodies are monoclonal antibodies.

30. Method for producing a preparation according to claim 28, characterized by the following steps:

- initiating an immune response in a non human animal by administering an antigen or a fragment thereof, as defined in any one of the claims 21 to 25, to said animal,
- removing the spleen or spleen cells from said animal,
- producing hybridoma cells of said spleen or spleen cells,
- selecting and cloning hybridoma cells specific for said anti-

gen and

•producing the antibody preparation by cultivation of said cloned hybridoma cells and optionally further purification steps.

31. Method according to claim 29, characterized in that said removing the spleen or spleen cells is connected with killing said animal.

32. Method for producing a preparation according to claim 27, characterized by the following steps:

•initiating an immune response in a non human animal by administering an antigen or a fragment thereof, as defined in any one of the claims 21 to 25, to said animal,

•removing an antibody containing body fluid from said animal,

•and

•producing the antibody preparation by subjecting said antibody containing body fluid to further purification steps.

33. Use of a preparation according to claim 27 or 28 for the manufacture of a medicament for treating or preventing staphylococcal infections or colonization in particular against Staphylococcus aureus or Staphylococcus epidermidis.

34. A screening method assessing the consequences of functional inhibition of at least one antigen or a fragment thereof, as defined in any one of claims 21 to 25.

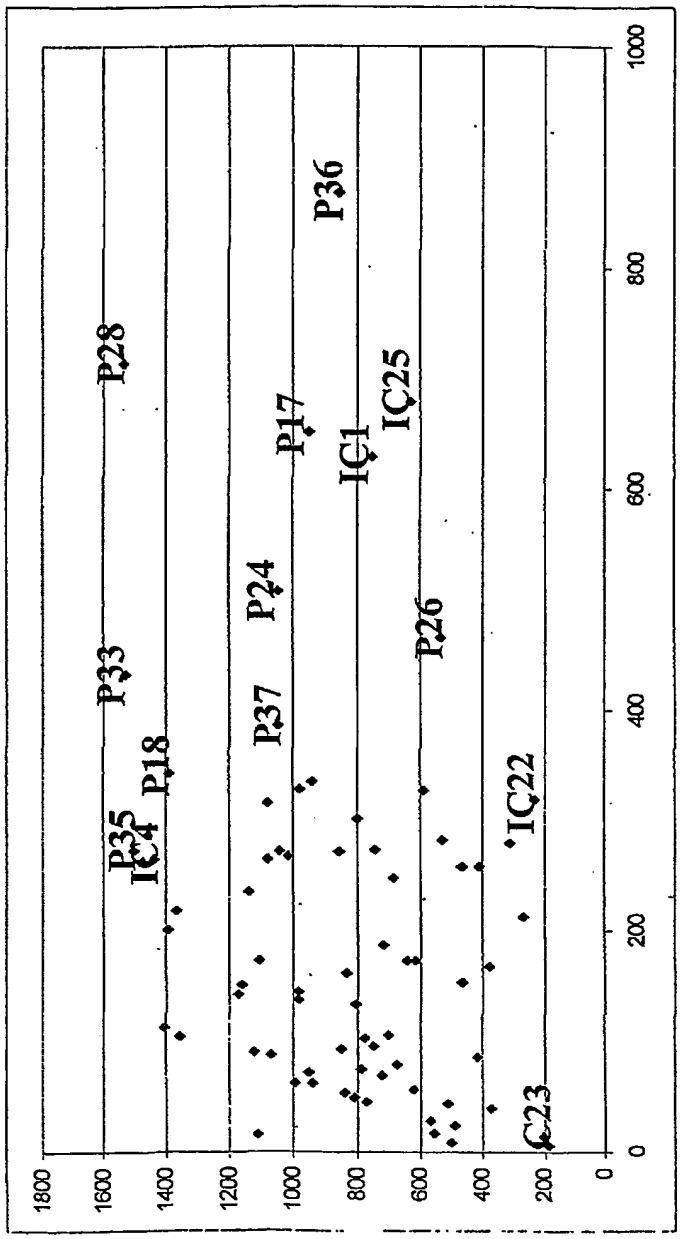
IgA vs. IgG titer against total S. aureus lysate
IgG titer 10.000X
IgA titer 10.000X
1800
1600
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
P28
P33
P35
IC4
P18
P37
P24
P17
P36
IC1
IC25
P26
IC22
IC23

Figure 1

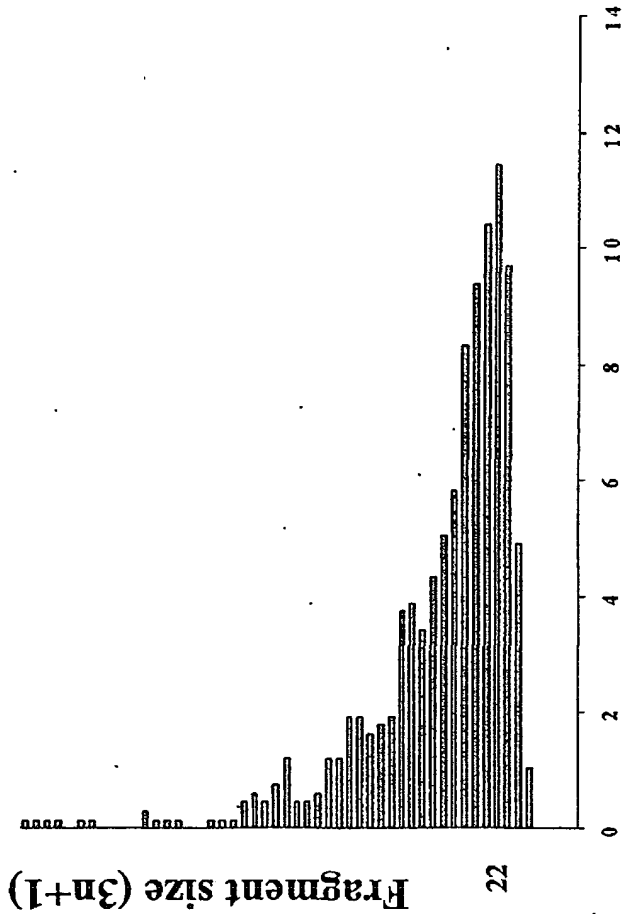

Figure 2

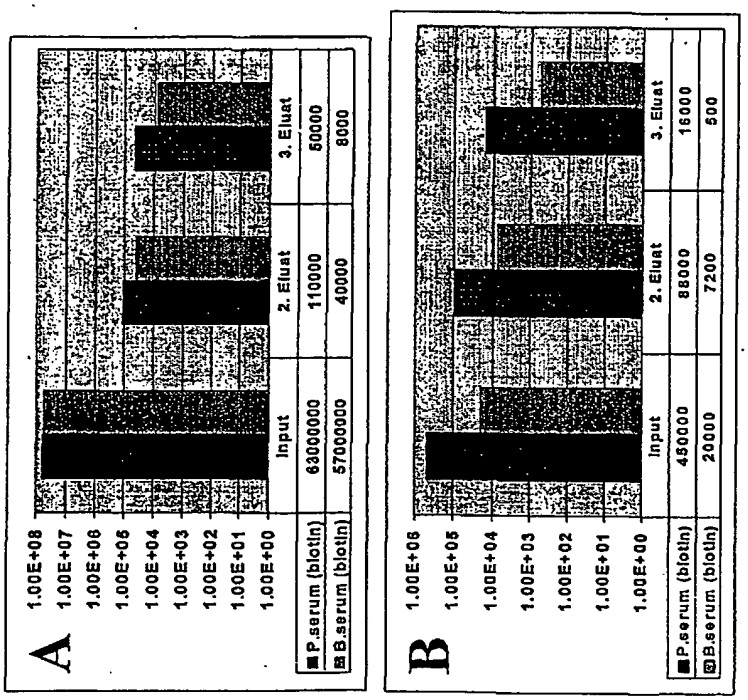
A
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Input
2. Eluat
3. Eluat
P.serum (biotin) 63000000 110000 50000
B.serum (biotin) 57000000 40000 8000
B
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
Input
2. Eluat
3. Eluat
P.serum (biotin) 450000 88000 16000
B.serum (biotin) 20000 7200 500

Figure 3

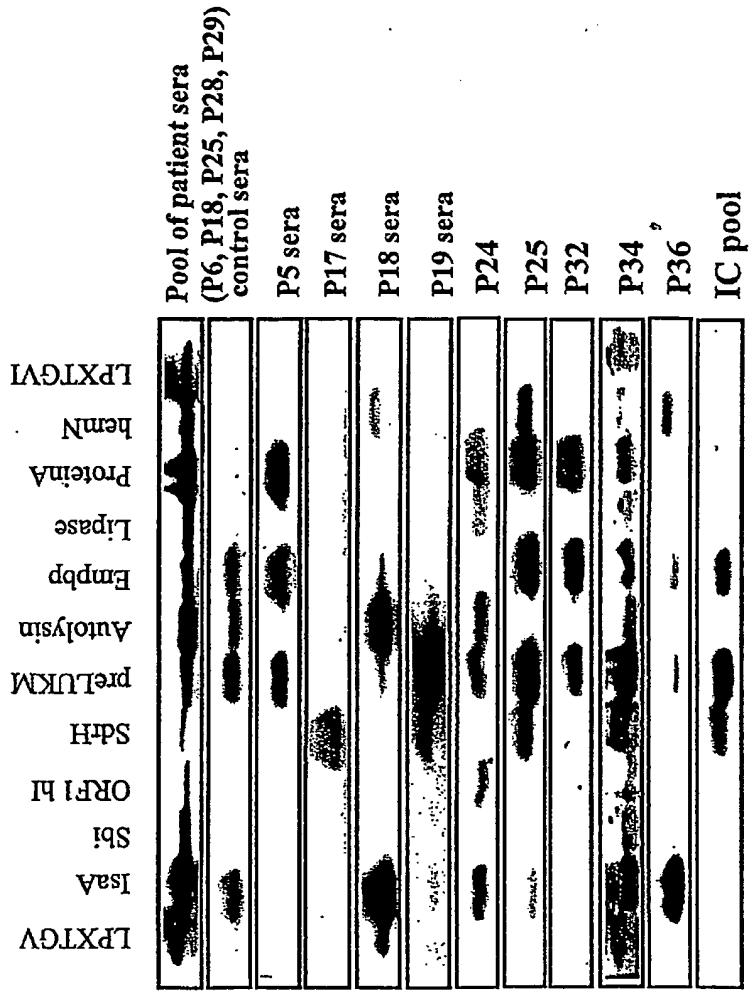
LPXTGV
IsaA
Sbi
ORF1 bl
SdrH
preLUKM
Autolysin
Empbp
Lipase
ProteinA
hemN
LPXTGVI
Pool of patient sera (P6, P18, P25, P28, P29) control sera
P5 sera
P17 sera
P18 sera
P19 sera
P24
P25
P32
P34
P36
IC pool

Figure 4

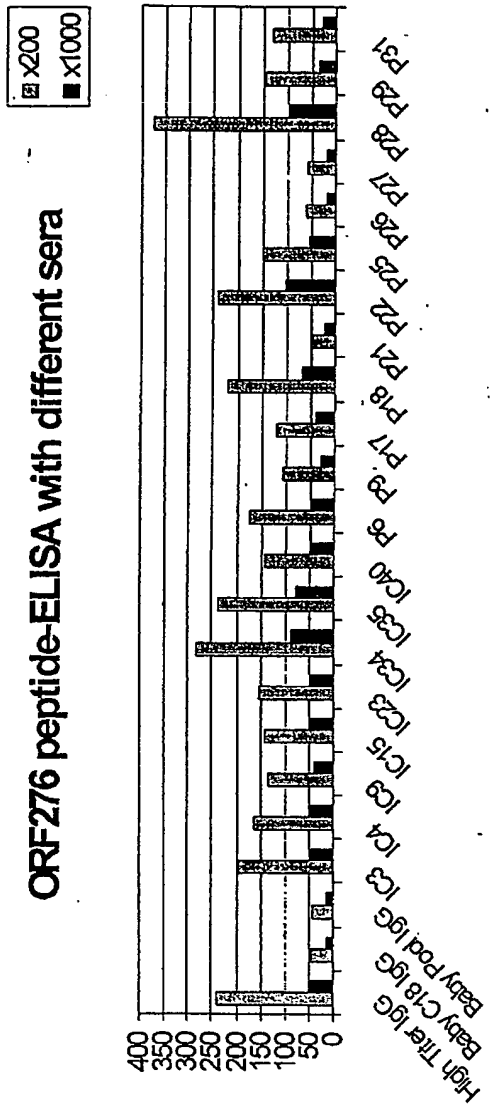
ORF276 peptide-ELISA with different sera
x200
x1000
400
350
300
250
200
150
100
50
0
High Titer IgG
Baby C18 IgG
Baby Pool IgG
IC3
IC4
IC9
IC15
IC23
IC34
IC35
IC40
P6
P9
P17
P18
P21
P22
P25
P26
P27
P28
P31

Figure 5

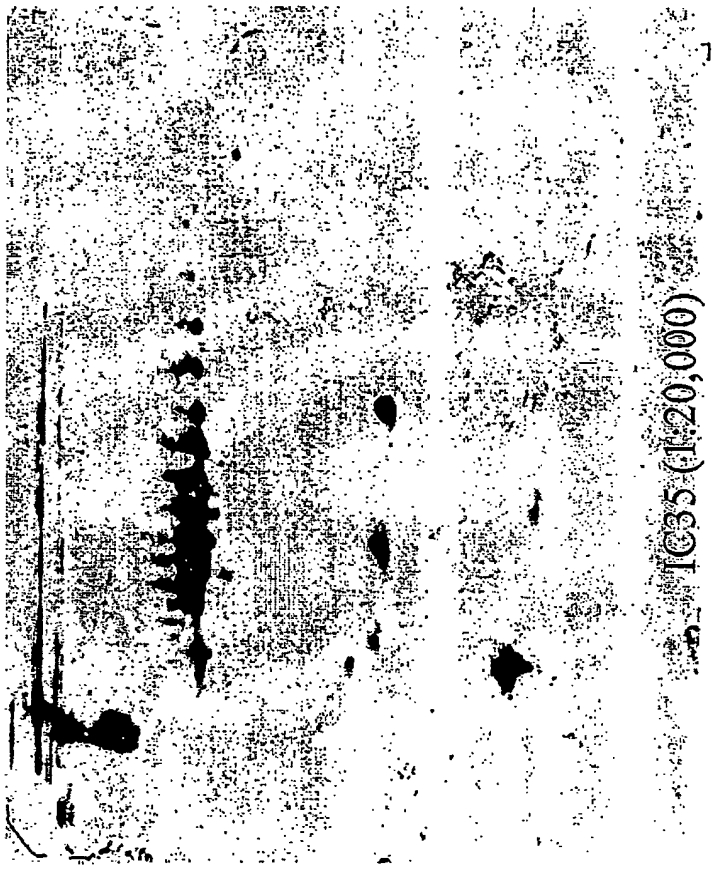

Figure 6

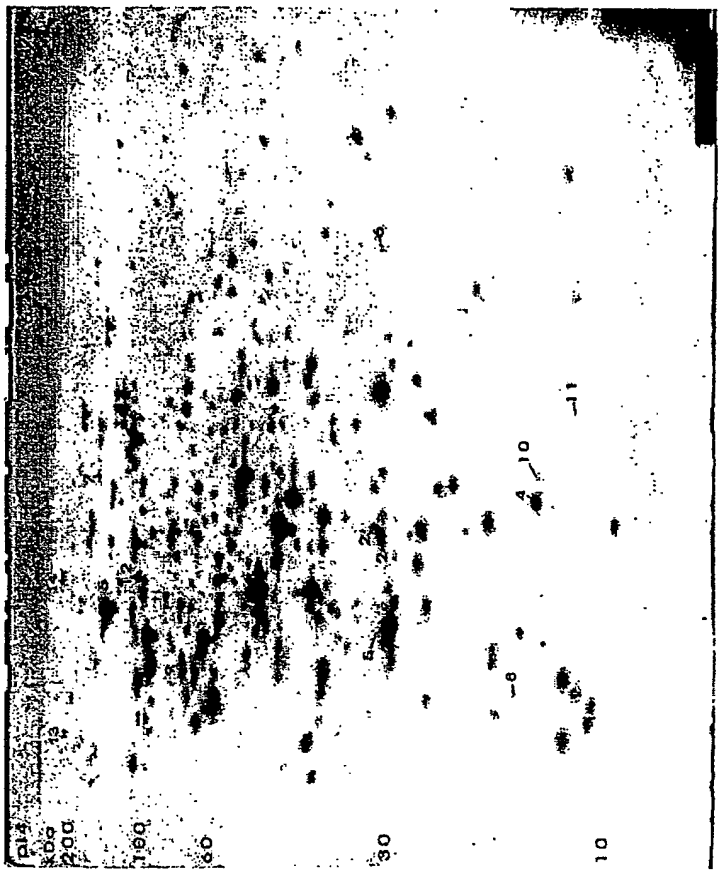

Figure 7

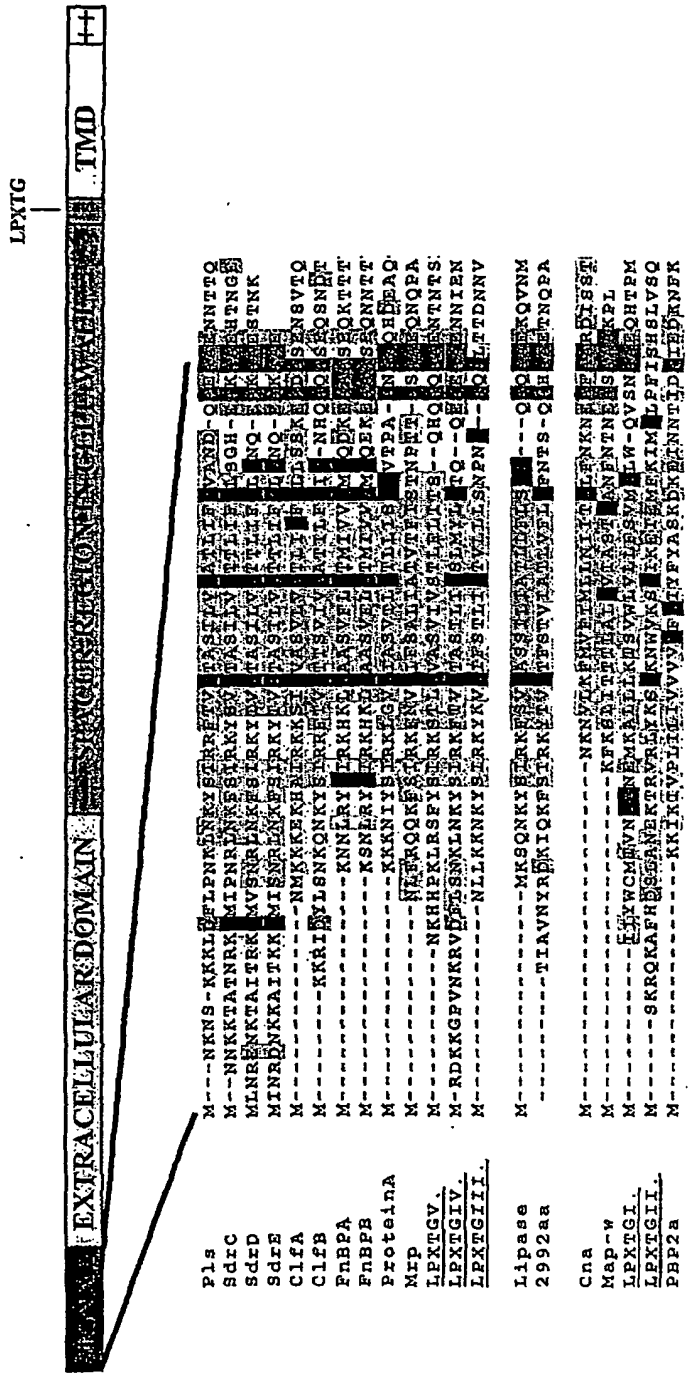
LPXTG
EXTRACELLULAR DOMAIN
TMD
Pls
SdrC
SdrD
SdrE
ClfA
ClfB
FnBPA
FnBPB
Protein A
Mrp
LPXTGV.
LPXTGIV.
LPXTGIII.
Lipase
2992aa
Cna
Map-w
LPXTGI.
LPXTGII.
PBP2a

Figure 8A

Constitutive Cell Wall Proteins of S. aureus with LPXTG motif

	Known proteins	Predicted Mw/pI		LPXTG / hydrophobic membrane domain basic C-terminus
1	Mrp protein	255/4.6		AKTLPDTGMSHNDDLFYAELALGAGMAFLIRRFTKKDQQTEE
2	Pls (MRSA)	167/4.1		NKELPDTGNDADNNGTLFGSLFAALGGLFLVGRRRKNKNNEEK
3	SdrD (SD-repeat)	133/4.1		AKALPETGNENSGSNNATLFGGLFAALGSLLLFGRRKKQNK
4	Cna	126/5.6		LKELPKTGMKQTITSWTLWVFIGIDGLYLLLRKRFNS
5	SdrE	117/4.1		AKALPETGSENNGSNNATLFGGLFAALGSLLLFGRRKKQNK
6	FnBPA	104/4.5		KSELPETGGEESTNKGMLFGGLFSILGLALLRRNKKNHKA
7	SdrC	94/4.1		AKALPETGSENNNSNNGTLFGGLFAALGSLLSEGRRKKQNK
8	FnBPB	96/4.5		KSELPETGGEESTNNGMLFGGLFSILGLALLRRNKKNHKA
9	ClfA (clumping facto	89/3.4		KEPLPDTGSEDEANTSLIWGLLASIGSLLLFRKKKENKDKK
10	ClfB (clumping facto	88/3.7		TDALPETGDKSENTNATLFGAMMALLGSLLLFRKRKQDHKEKA
11	Spa (Protein A)	48/5.2		AQALPETGEENPFIGTTVFGGLSLALGAALLAGRRREL

	Predicted based on sequence (TIGR)			
1	Anonymus I.	79/9.3		EKQLPKTGTNKSSSPEAMFVLLAGIGLIATVRRRKAS
2	Anonymus II.	227/4.2		EKRLPDTGDSIKQNGLLGGVMTLLVGLGLMKRKKKKDEND
3	Anonymus III.	200/4.1		EKELPNTGSEGMDLPLKDFALITGAAILARRRTKNEKES
4	Anonymus IV.	122/5.8		RAELPKTGLESTQKGLIFSSIIGIAGLMLIARRRKN
5	Anonymus V.	101/5.0		SKMLPKTGETTSSQSWWGLYALLGMLALFIPKFRKESK

Figure 8B

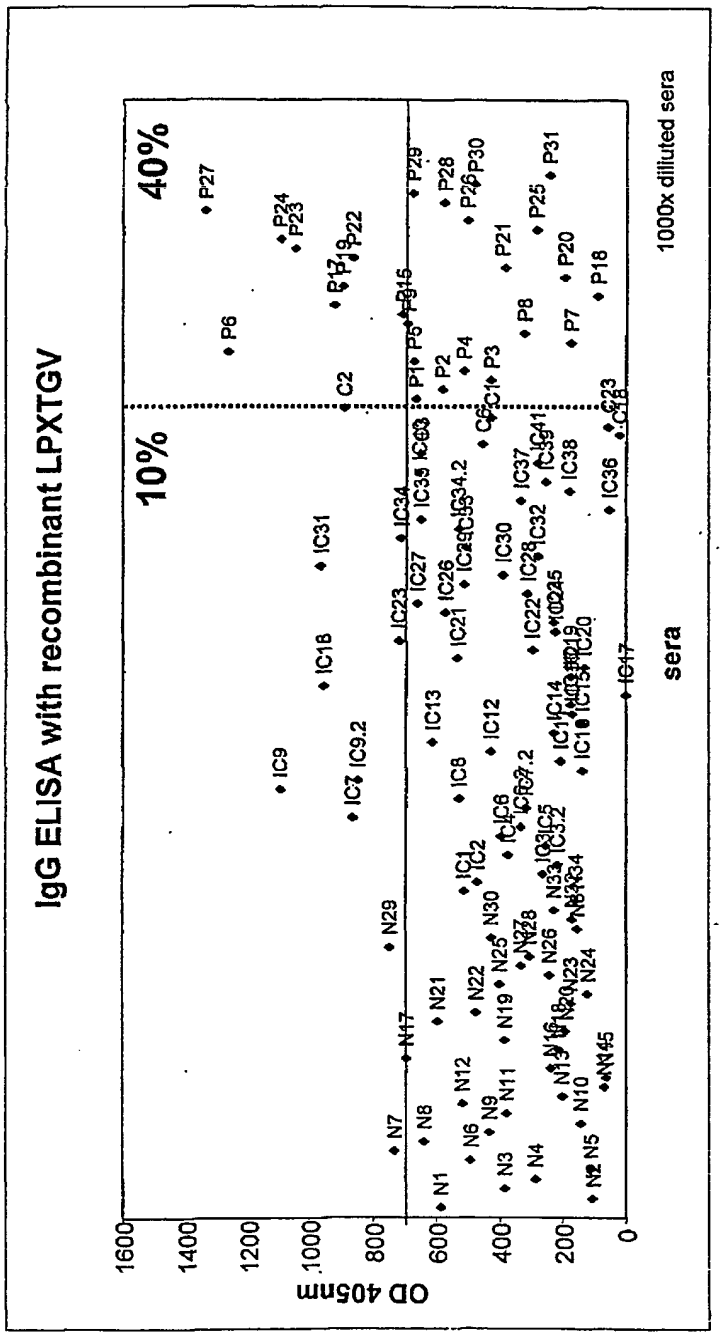
IgG ELISA with recombinant LPXTGV
10%
40%
OD 405nm
1600
1400
1200
1000
800
600
400
200
0
sera
1000x diluted sera

Figure 9

Surface staining of S. aureus (strain 8325-4 spa-) with purified anti-LPXTGV IgGs

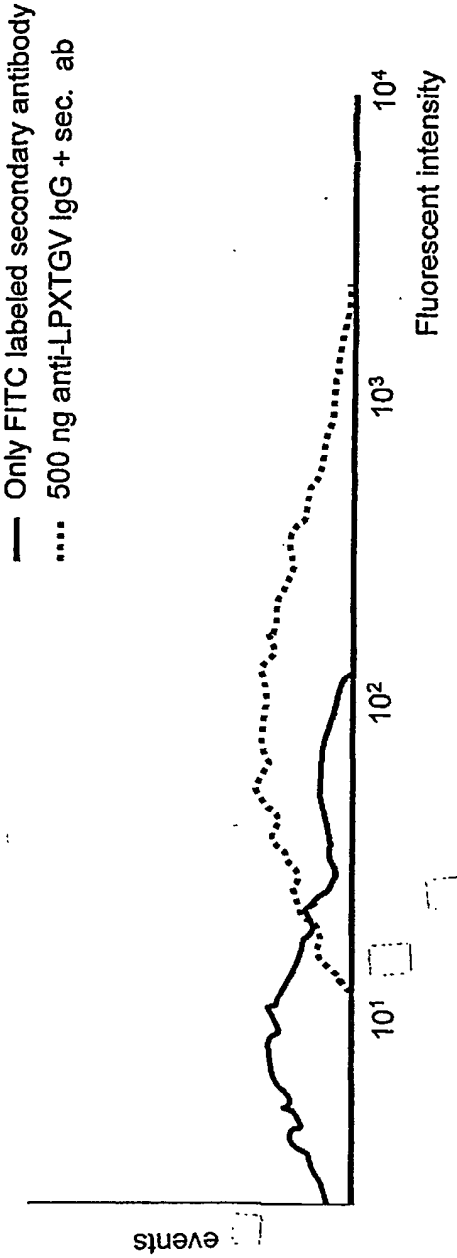

Figure 10

SEQUENCE LISTING

Intercell Biomedizinische Forschungs- und Entwicklungs AG
Cistem Biotechnologies GmbH

R 39035
Priority: Austrian Patent Application No. A 130/2001 of 26.01.2001

Seq.ID Nos. 1-598
Organisms: S.aureus; S.epidermidis

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19.

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29.

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42.

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47.
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48.
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49.
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54.

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56.

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57.

Msdfnhtdhsttnhsqtpryrrpkfpwfktvivaliagiigallvlgigkvlnstilnkd
Gstvqttnnkggnqldgqskkfgtvhemiksvsptivgvinmqkassvddllkgksskps
Eagvgsgviyqinnnsayivtnnhvidganeirvqlhnkkqvkaklvgkdavtdiavlki
Entkgikaiqfansskvqtgdsvfamgnplglqfansvtsgiisasertidaettggntk
Vsvlqtdaainpgnsggalvdingnlvginsmkiaatqvegigfaipsnevkvtieqlvk
Hgkidrpsigiglinlkdipeeereqlhtdredgiyvakadsdidlkkgdiiteidgkki
Kddvdlrsylyenkkpgesvtvtvirdgktkevkvklkqqkeqpkrqsrserqspgqgdr
dffr

58.

VNQQQEKTTTTPTTINPLTGEKVGEGEPTTEVTKEPVDEITQFGGEEVPQGHKDEFDPNL
PIDGTEEVPGKPGIKNPETGEVVTPPVDDVTKHGPKAGEPEVTKEEIPFEKKREFNPDLK
PGEEKVTQEGQTGEKTTTTPTTINPLTGEKVGEGEPTTEVTKEPVDEITQFGGEEVPQGH
KDEFDPNLPIDGTEEVPGKPGIKNPETGEVVTPPVDDVTKHGPKAGEPEVTKEEIPYETK
RVLDPTMEPGSPDKVAQKGENGEKTTTTPTTINPLTGEKVGEGEPTTEVTKEPIDEIVNY
APEIIPHGTREEIDPNLPEGETKVIPGKDGLKDPETGEIIEEPQDEVIIHGAKDDSDADS
DSDADSDSDADSDSDADSDSDADSDSDSDSDSDSDSDADSDSDADSDSDSDSDADS
DSDADSDSDADSDSDADSDSDSDSDADSDSDSDADSDSDADSDSDADS
DSDSDSDSDADSDSDSDSDSDADSDSDSDADSDSDADSDSDADSDSDADSDSDS
DSDSDSDSDSDSDRDSDADSDSDADSDSDSDSDADSDSDSDSDADSDSDADSDSDS
DADSDSDSDSDSDADSDSDADSDSDADSDSDSDSDSDSDADSDS
DADRDHNDKTDKPNNKELPDTGNDAQNNGTLFGSLFAALGGLFLVGRRRKNKNNEEK

59.

MKSLKTVIGMNNKEHIKSVILALLVLMSVVLTYMVWNFSPDIANVDNTDSKKSETKPLTT
PMTAKMDTTITPFQIIHSKNDHPEGTIATVSNVNKLTKPLKNKEVKSVEHVRRDHNLMIP
DLNSDFILFDFTYDLPLSTYLGQVLNMNAKVPNHFNFNRLVIDHDADDNIVLYAISKDRH
DYVKLTTTTKNDHFLDALAAVKKDMQPYTDIITNKDTIDRTTHVFAPSKPEKLKTYRMVF
NTISVEKMNAILFDDSTIVRSSKSGVTTYNNNTGVANYNDKNEKYHYKNLSEDEASSSKM
EETIPGTFDFINGHGGFLNEDFRLFSTNNQSGKLTYQRFLNGYPTFNKEGSNQIQVTWGE
KGVFDYRRSLLRTDVVLNSEDNKSLPKLESVRSSLANNSDINFEKVTNIAIGYEMQDNSD
HNHIEVQINSELVPRWYVEYDGEWYVYNDGRLE

60.	MSKRQKAFHDSLANEKTRVRLYKSGKNWVKSGIKEIEMFKIMGLPPISHSLVSQDNQSIS KKMTGYGLKTTAVIGGAFTVNMLHDQQAFAASDAPLTSELNTQSETVGNQNSTTIEASTS TADSTSVTKNSSSVQTSNSDTVSSEKSEKVTSTTNSTSNQQEKLTSTSESTSSKNTTSSS DTKSVASTSSTEQPINTSTNQSTASNNTSQSTTPSSVNLNKTSTTSTSTAPVKLRTFSRL AMSTFASAATTTAVTANTITVNKDNLKQYMTTSGNATYDQSTGIVTLTQDAYSQKGAITL GTRIDSNKSFHFSGKVNLGNKYEGHGNGGDGIGFAFSPGVLGETGLNGAAVGIGGLSNAF GFKLDTYHNTSKPNSAAKANADPSNVAGGGAFGAFVTTDSYGVATTYTSSSTADNAAKLN VQPTNNTFQDFDINYNGDTKVMTVKYAGQTWTRNISDWIAKSGTTNFSLSMTASTGGATN LQQVQFGTFEYTESAVTQVRYVDVTTGKDIIPPKTYSGNVDQVVTIDNQQSALTAKGYNY TSVDSSYASTYNDTNKTVKMTNAGQSVTYYFTDVKAPTVTVGNQTIEVGKTMNPIVLTTT DNGTGTVTNTVTGLPSGLSYDSATNSIIGTPTKIGQSTVTVVSTDQANNKSTTTFTINVV DTTAPTVTPIGDQSSEVYSPISPIKIATQDNSGNAVTNTVTGLPSGLTFDSTNNTISGTP TNIGTSTISIVSTDASGNKTTTTFKYEVTRNSMSDSVSTSGSTQQSQSVSTSKADSQSAS TSTSGSIVVSTSASTSKSTSVSLSDSVSASKSLSTSESNSVSSSTSTSLVNSQSVSSSMS DSASKSTSLSDSISNSSSTEKSESLSTSTSDSLRTSTSLSDSLSMSTSGSLSKSQSLSTS ISGSSSTSASLSDSTSNAISTSTSLSESASTSDSISISNSIANSQSASTSKSDSQSTSIS LSTSDSKSMSTSESLSDSTSTSGSVSGSLSIAASQSVSTSTSDSMSTSEIVSDSISTSGS LSASDSKSMSVSSSMSTSQSGSTSESLSDSQSTSDSDSKSLSQSTSQSGSTSTSTSTSAS VRTSESQSTSGSMSASQSDSMSISTSFSDSTSDSKSASTASSESISQSASTSTSGSVSTS TSLSTSNSERTSTSMSDSTSLSTSESDSISESTSTSDSISEAISASESTFISLSESNSTS DSESQSASAFLSESLSESTSESTSESVSSSTSESTSLSDSTSESGSTSTSLSNSTSGSTS ISTSTSISESTSTFKSESVSTSLSMSTSTSLSDSTSLSTSLSDSTSDSKSDSLSTSMSTS DSISTSKSDSISTSTSLSGSTSESKSDSTSMSISMSQSTSGSTSTSTSTSLSDSTSTSLS LSASMNQSGVDSNSASQSASNSTSTSTSESDSQSTSSYTSQSTSQSESTSTSTSLSDSTS ISKSTSQSGSVSTSASLSGSESESDSQSISTSASESTSESASTSLSDSTSTSNSGSASTS TSLSNSASASESDLSSTSLSDSTSASMQSSESDSQSTSASLSDSLSTSTSNRMSTIASLS TSVSTSESGSTSESTSESDSTSTSLSDSQSTSRSTSASGSASTSTSTSDSRSTSASTSTS MRTSTSDSQSMSLSTSTSTSMSDSTSLSDSVSDSTSDSTSASTSGSMSVSISLSDSTSTS TSASEVMSASISDSQSMSESVNDSESVSESNSESDSKSMSGSTSVSDSGSLSVSTSLRKS ESVSESSSLSCSQSMSDSVSTSDSSSLSVSTSLRSSESVSESDSLSDSKSTSGSTSTSTS GSLSTSTSLSGSESVSESTSLSDSISMSDSTSTSDSDSLSGSISLSGSTSLSTSDSLSDS KSLSSSQSMSGSESTSTSVSDSQSSSTSNSQFDSMSISASESDSMSTSDSSSISGSNSTS TSLSTSDSMSGSVSVSTSTSLSDSISGSTSVSDSSSTSTSTSLSDSMSQSQSTSTSASGS LSTSISTSMSMSASTSSSQSTSVSTSLSTSDSISDSTSISISGSQSTVESESTSDSTSIS DSESLSTSDSDSTSTSTSDSTSGSTSTSISESLSTSGSGSTSVSDSTSMSESNSSSVSMS QDKSDSTSISDSESVSTSTSTSLSTSDSTSTSESLSTSMSGSQSISDSTSTSMSGSTSTS ESNSMHPSDSMSMHHTHSTSTSRLSSEATTSTSESQSTLSATSEVTKHNGTPAQSEKRLP DTGDSIKQNGLLGGVMTLLVGLGLMKRKKKKDENDQDDSQA
61.	MPKNKILIYLLSTTLVLPTLVSPTAYADTPQKDTTAKTTSHDSKKSNDDETSKDTTSKDI DKADKNNTSNQDNNDKKFKTIDDSTSDSNNIIDFIYKNLPQTNINQLLTKNKYDDNYSLT TLIQNLFNLNSDISDYEQPRNGEKSTNDSNKNSDNSIKNDTDTQSSKQDKADNQKAPKSN NTKPSTSNKQPNSPKPTQPNQSNSQPASDDKANQKSSSKDNQSMSDSALDSILDQYSEDA KKTQKDYASQSKKDKNEKSNTKNPQLPTQDELKHKSKPAQSFNNDVNQKDTRATSLFETD PSISNNDDSGQFNVVDSKDTRQFVKSIAKDAHRIGQDNDIYASVMIAQAILESDSGRSAL AKSPNHNLFGIKGAFEGNSVPFNTLEADGNQLYSINAGFRKYPSTKESLKDYSDLIKNGI DGNRTIYKPTWKSEADSYKDATSHLSKTYATDFNYAKKLNSIIKHYQLTQFDDERMPDLD KYERSIKDYDDSSDEFKPFREVSDSMPYPHGQCTWYVYNRMKQFGTSISGDLGDAHNWNN RAQYRDYQVSHTPKRHAAVVFEAGQFGADQHYGHVAFVEKVNSDGSIVISESNVKGLGII SHRTINAAAAEELSYITGK
62.	MRKFSRYAFTSMAALTLLSTLSPAALAIDSKNKPANSDIKFEVTQKSDAVKALKELPKSE NVKNIYQDYAVTDVKTDKKGFTHYTLQPSVDGVHAPDKEVKVHADKSGKVVLINGDTDAK KVKPTNKVTLSKDDAADKAFKAVKIDKNKAKNLKDKVIKENKVEIDGDSNKYVYNVELIT VTPEISHWKVKIDAQTGEILEKMNLVKEAAETGKGKGVLGDTKDININSIDGGFSLEDLT HQGKLSAFSFNDQTGQATLITNEDENFVKDEQRAGVDANYYAKQTYDYYKDTFGRESYDN QGSPIVSLTHVNNYGGQDNRNNAAWIGDKMIYGDGDGRTFTSLSGANDVVAHELTHGVTQ ETANLEYKDQSGALNESFSDVFGYFVDDEDFLMGEDVYTPGKEGDALRSMSNPEQFGQPA HMKDYVFTEKDNGGVHTNSGIPNKAAYNVIQAIGKSKSEQIYYRALTEYLTSNSNFKDCK DALYQAAKDLYDEQTAEQVYEAWNEVGVE
63.	MKKRIDYLSNKQNKYSIRRFTVGTTSVIVGATILFGIGNHQAQASEQSNDTTQSSKNNAS ADSEKNNMIETPQLNTTANDTSDISANTNSANVDSTTKPMSTQTSNTTTTEPASTNETPQ PTAIKNQATAAKMQDQTVPQEANSQVDNKTTNDANSIATNSELKNSQTLDLPQSSPQTIS NAQGTSKPSVRTRAVRSLAVAEPVVNAADAKGTNVNDKVTASNFKLEKTTFDPNQSGNTF MAANFTVTDKVKSGDYFTAKLPDSLTGNGDVDYSNSNNTMPIADIKSTNGDVVAKATYDI LTKTYTFVFTDYVNNKENINGQFSLPLFTDRAKAPKSGTYDANINIADEMFNNKITYNYS SPIAGIDKPNGANISSQIIGVDTASGQNTYKQTVFVNPKQRVLGNTWVYIKGYQDKIEES SGKVSATDTKLRIFEVNDTSKLSDSYYADPNDSNLKEVTDQFKNRIYYEHPNVASIKPGD ITKTYVVLVEGHYDNTGKNLKTQVIQENVDPVTNRDYSIFGWNNENVVRYGGGSADGDSA VNPKDPTPGPPVDPEPSPDPEPEPTPDPEPSPDPEPEPSPDPDPDSDSDSGSDSDSGS DSDSESDSDSDSDSDSDSDSDSESDSDSESDSDSDSDSDSDSDSDSDSDSDSDSDSDS DSDSESDSDSESDSESDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDS DSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDS DSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDS RVTPPNNEQKAPSNPKGEVNHSNKVSKQHKTDALPETGDKSENTNATLFGAMMALLGSLL LFRKRKQDHKEKA
64.	MKKTIMASSLAVALGVTGYAAGTGHQAHAAEVNVDQAHLVDLAHNHQDQLNAAPIKDGAY DIHFVKDGFQYNFTSNGTTWSWSYEAANGQTAGFSNVAGADYTTSYNQGSNVQSVSYNAQ SSNSNVEAVSAPTYHNYSTSTTSSSVRLSNGNTAGATGSSAAQIMAQRTGVSASTWAAII ARESNGQVNAYNPSGASGLFQTMPGWGPTNTVDQQINAAVKAYKAQGLGAWGP
65.	MGGYLIMKKIVTATIATAGLATIAPAGHDAQAAEQNNNGYNSNDAQSYSYTYTIDAQGNY HYTWTGNWNPSQLTQNNTYYYNNYNTYSYNNASYNNYYNHSYQYNNYTNNSQTATNNYYT GGSGASYSTTSNNVHVTTTAAPSSNGRSISNGYASGSNLYTSGQCTYYVFDRVGGKIGST WGNASNWANAAASSGYTVNNTPKVGAIMQTTQGYYGHVAYVEGVNSNGSVRVSEMNYGHG AGVVTSRTISANQAGSYNFIH

66.	MANTKKTTLDITGMTCAACSNRIEKKLNKLDDVNAQVNLTTEKATVEYNPDQHDVQEFIN TIQHLGYGVAVETVELDITGMTCAACSSRIEKVLNKMDGVQNATVNLTTEQAKVDYYPEE TDADKLVTRIQKLGYDASIKDNMKDQTSRKAEALQHKLIKLIISAVLSLPLLMLMFVHLF NMHIPALFTNPWFQFILATPVQFIIGWQFYVGAYKNLRNGGANMDVLVAVGTSAAYFYSI YEMVRWLNGSTTQPHLYFETSAVLITLILFGKYLEARAKSQTTNALGELLSLQAKEARIL KDGNEVMIPLNEVHVGDTLIVKPGEKIPVDGKIIKGMTAIDESMLTGESIPVEKNVDDTV IGSTMNKNGTITMTATKVGGDTALANIIKVVEEAQSSKAPIQRLADIISGYFVPIVVGIA LLTFIVWITLVTPGTFEPALVASISVLVIACPCALGLATPTSIMVGTGRAAENGILFKGG EFVERTHQIDTIVLDKTGTITNGRPVVTDYHGDNQTLQLLATAEKDSEHPLAEAIVNYAK EKQLILTETTTFKAVPGHGIEATIDHHHILVGNRKLMADNDISLPKHISDDLTHYERDGK TAMLIAVNYSLTGIIAVADTVKDHAKDAIKQLHDMGIEVAMLTGDNKNTAQAIAKQVGID TVIADILPEEKAAQIAKLQQQGKKVAMVGDGVNDAPALVKADIGIAIGTGTEVAIEAADI TILGGDLMLIPKAIYASKATIRNIRQNLFWAFGYNIAGIPIAALGLLAPWVAGAAMALSS VSVVTNALRLKKMRLEPRRKDA
67.	MFDSIRETIDYAVENNMSFADIMVKEEMELSGKSRDEVRAQMKQNLDVMRDAVIKGTTGD GVESVTGYTGHDAAKLRDYNETHHALSGYEMIDAVKGAIATNEVNAAMGIICATPTAGSS GTIPGALFKLEKTHDLTEEQMIDFLFTSALFGRVVANNASVAGATGGCQAEVGSASAMAA AAAVAIFGGSPEASGHAMALAISNLLGLVCDPVAGLVEIPCVMRNAIGSGNALISADLAL AGIESRIPVDEVIEAMDKVGRNLPASLRETGLGGLAGTPTGEAIKRKIFGTAEDMVKNN
68.	MKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEAAASEQKTTTVEENGNSATDNKTSET QTTATNVNHIEETQSYNATVTEQPSNATQVTTEEAPKAVQAPQTAQPANIETVKEEVVKE EAKPQVKETTQSQDNSGDQRQVDLTPKKATQNQVAETQVEVAQPRTASESKPRVTRSADV AEAKEASNAKVETGTDVTSKVTVEIGSIEGHNNTNKVEPHAGQRAVLKYKLKFENGLHQG DYFDFTLSNNVNTHGVSTARKVPEIKNGSVVMATGEVLEGGKIRYTFTNDIEDKVDVTAE LEINLPIDPKTVQTNGNQTITSTLNEEQTSKELDVKYKDGIGNYYANLNGSIETFNKANN RFSHVAFIKPNNGKTTSVTVTGTLMKGSNQNGNQPKVRIFEYLGNNEDIAKSVYANTTDT SKFKEVTSNMSGNLNLQNNGSYSLNIENLDKTYVVHYDGEYLNGTDEVDFRTQMVGHPEQ LYKYYYDRGYTLTWDNGLVLYSNKANGNGKNGPIIQNNKFEYKEDTIKETLTGQYDKNLV TTVEEEYDSSTLDIDYHTAIDGGGGYVDGYIETIEETDSSAIDIDYHTAVDSEAGHVGGY TESSEESNPIDFEESTHENSKHHADVVEYEEDTNPGGGQVTTESNLVEFDEESTKGIVTG AVSDHTTVEDTKEYTTESNLIELVDELPEEHGQAQGPVERITENNHHISHSGLGTENGHG NYDVIERIKENSHVDIKSELGYEGGQNSGNQSFEEDTEEDKPKYEQGGNIVDIDFDSVPQ IHGQNRGNQSFEEDTEKDKPKYEHGGNIIDIDFDSVPHIHGFNKHTEIIEEDTNKDKPSY QFGGHNSVDFEEDTLPKVSGQNEGQQTIEEDTTPPIVPPTPPTPEVPSEPETPTPPTPEV PSEPETPTPPTPEVPSEPETPTPPTPEVPAEPGKPVPPAKEEPKKPSKPVEQGKVVTPVI EINEKVKAVAPTKKPQSKKSELPETGGEESTNKGMLFGGLFSILGLALLRRNKKNHKA
69.	LHLRENIIVKSNLRYGIRKHKLGAASVFLGTMIVVGMGQEKEAAASEQNNTTVEESGSSA TESKASETQTTTNNVNTIDETQSVSATSTEQPSQSTQVTTEEAPKTVQAPKVETSRVDLP SEKVADKETTGTQVDIAQPSNVSEIKPRMKRSTDVTAVAEKEVVEETKATGTDVTNKVEV EEGSEIVGHKQDTNVVNPHNAERVTLKYKWKFGRGIKAGDYFDFTLSDNVETHGISTLRK VPEIKSTDGQVMATGEIIGERKVRYTFKEYVQEKKDLTAELSLNLFIDPTTVTQKGNQNV EVKLGETTVSKIFNIQYLGGVRDNWGVTANGRIDTLNKVDGKFSHFAYMKPNNQSLSSVT VTGQVTKGNKPGVNNPTVKVYKHIGSDDLAESVYAKLDDVSKFEDVTDNMSLDFDTNGGY SLNFNNLDQSKNYVIKYEGYYDSNASNLEFQTHLFGYYNYYYTSNLTWKNGVAFYSNNAQ GDGKDKLKEPIIEHSTPIELEFKSEPPVEKHELTGTIEESNDSKPIDFEYHTAVEGAEGH AEGTIETEEDSIHVDFEESTHENSKHHADVVEYEEDTNPGGGQVTTESNLVEFDEDSTKG IVTGAVSDHTTIEDTKEYTTESNLIELVDELPEEHGQAQGPIEEITENNHHISHSGLGTE NGHGNYGVIEEIEENSHVDIKSELGYEGGQNSGNQSFEEDTEEDKPKYEQGGNIVDIDFD SVPQIHGQNNGNQSFEEDTEKDKPKYEQGGNIIDIDFDSVPHIHGFNKHTEIIEEDTNKD KPNYQFGGHNSVDFEEDTLPQVSGHNEGQQTIEEDTTPPIVPPTPPTPEVPSEPETPTPP TPEVPSEPETPTPPTPEVPTEPGKPIPPAKEEPKKPSKPVEQGKVVTPVIEINEKVKAVV PTKKAQSKKSELPETGGEESTNNGMLFGGLFSILGLALLRRNKKNHKA
70.	MQMRDKKGPVNKRVDFLSNKLNKYSIRKFTVGTASILIGSLMYLGTQQEAEAAENNIENP TTLKDNVQSKEVKIEEVTNKDTAPQGVEAKSEVTSNKDTIEHEPSVKAEDISKKEDTPKE VADVAEVQPKSSVTHNAETPKVRKARSVDEGSFDITRDSKNVVESTPITIQGKEHFEGYG SVDIQKKPTDLGVSEVTRFNVGNESNGLIGALQLKNKIDFSKDFNFKVRVANNHQSNTTG ADGWGFLFSKGNAEEYLTNGGILGDKGLVNSGGFKIDTGYIYTSSMDKTEKQAGQGYRGY GAFVKNDSSGNSQMVGENIDKSKTNFLNYADNSTNTSDGKFHGQRLNDVILTYVASTGKM RAEYAGKTWETSITDLGLSKNQAYNFLITSSQRWGLNQGINANGWMRTDLKGSEFTFTPE APKTITELEKKVEEIPFKKERKFNPDLAPGTEKVTREGQKGEKTITTPTLKNPLTGVIIS KGEPKEEITKDPINELTEYGPETIAPGHRDEFDPKLPTGEKEEVPGKPGIKNPETGDVVR PPVDSVTKYGPVKGDSIVEKEEIPFXKERKFNPDLAPGTEKVTREGQKGEKTITTPTLKN PLTGEIISKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEEVPGKPGIKN PETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFKKERKFNPDLAPGTEKVTREGQKGEKT ITTPTLKNPLTGEIISKGESKEEITKDPINELTEYGPETITPGHRDEFDPKLPTGEKEEV PGKPGIKNPETGDVVRPPVDSVTKYGPVKGDSIVEKEEIPFEKERKFNPDLAPGTEKVTR EGQKGEKTITTPTLKNPLTGEIISKGESKEEITKDPVNELTEPGGEKIPQGHKDIFDPNL PTDQTEKVPGKPGIKNPDTGKVIEEPVDDVIKHGPKTGTPETKTVEIPFETKREFNPKLQ PGEERVKQEGQPGSKTITTPITVNPLTGEKVGEGQPTEEITKPQVDKIVEFGGEKPKDPK GPENPEKPSRPTHPSGPVNPNNPGLSKDRAKPNGPVHSMDKNDKVKKSKIAKESVANQEK KRAELPKTGLESTQKGLIPSSIIGIAGLMLLARRRKN
71.	MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN SKLPKDLRDKNNRFVEKVSIEKAIVRHDERRVKSANDAISKLNEKDSIENRRLAQREVNKA PMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKVESPKVEVPQIQSPKVE VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDQTVLTVL GSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQET ASSIKNTLSNLLSFWK
72.	MAVFSKEKKRGCIVVIETFKAFVIDKDESGKVTPTFKQLSPTDLPKGDVLIKVHYSGINY KDALATQDHNAVVKSYPMIPGIDLAGTIVESEAPGFEKGEQVIVTSYDLGVSHYGGFSEY ARVKSEWIIKLPDTLTLEESMIYGTAGYTAGLAIERLEKVGMNIEDGPVLVRGASGGVGT LAVLMLNELGYKVIASTGKQDVSDQLLELGAKEVIDRLPVEDDHKKPLASSTWQACVDPV GGEGINYVTKRLNHSGSIAVIGMTAGNTYTNSVFPHILRGVNILGIDSVFTAMKLRQRVW RRLAKDLMPENLHEIKQVITFDELPEQLNKVIKHENKGRIVIDFGVDK
73.	MKKLVTATTLTAGIGTALVGQAYHADAAENYTNYNNYNYNTTQTTTTTTTTTTTSSISHS GNLYTAGQCTWYVYDKVGGEIGSTWGNANNWAAAAQGAGFTVNHTPSKGAILQSSEGPFG HVAYVESVNSDGSVTISEMNYSGGPFSVSSRTISASEAGNYNYIHI

74.	MKKIATATIATAGFATIAIASGNQAHASEQDNYGYNPNDPTSYSYTYTIDAQGNYHYTWK GNWHPSQLNQDNGYYSYYYYNGYNNYNNYNNGYSYNNYSRYNNYSNNNQSYNYNNYNSYN TNSYRTGGLGASYSTSSNNVQVTTTMAPSSNGRSISSGYTSGRNLYTSGQCTYYVFDRVG GKIGSTWGNASNWANAAARAGYTVNNTPKAGAIMQTTQGAYGHVAYVESVNSNGSVRVSE MNYGYGPGVVTSRTISASQAAGYNFIH
75.	MSMTYRIKKWQKLSTITLLMAGVITLNGGSFRSVDKHQIAVADTNVQTPDYEKLRNTWLD VNYGYDKYDENNPDMKKKFDATEKRATNLLKEMKTESGRKYLWSGAETLETNSSHMTRTY RNIEKIAEAMRNPKTTLNTDENKKKVKDALEWLHKNAYGKEPDKKVKELSENFTKTTGKN TNLNWWDYEIGTPKSLTNTLILLNDQFSNEEKKKFTAPIKTFAPDSDKILSSVGKAELAK GGNLVDISKVKLLECIIEEDKDMMKKSIDSFNKVFTXVQDSATGKERNGFYKDGSYIDHQ DVPYTGAYGVVLLEGISQMMPMIKETPFNDKTQNDTTLKSWIDDGFMPLIYKGEMMDLSR GRAISRENETSHSASATVMKSLLRLSDAMDDSTKAKYKKIVKSSVESDSSYKQNDYLNSY SDIDKMKSLMTDNSISKNGLTQQLKIYNDMDRVTYHNKDLDFAFGLSMTSKNVARYESIN GENLKGWHTGAGMSYLYNSDVKHYHDNFWVTADMKRLSGTTTLDNEILKDTDDKKSSKTF VGGTKVDDQHASIGMDFENQDKTLTAKKSYFILNDKIVFLGTGIKSTDSSKNPVTTIENR KANGYTLYTDDKQTTNSDNQENNSVFLESTDTKKNIGYHFLNKPKITVKKESHTGKWKEI NKSQKDTQRTDEYYEVTQKHSNSDNKYGYVLYPGLSKDVFKTKKDEVTVVKQEDDFHVVK DNESVWAGVNYSNSTQTFDINNTKVEVKAKGMFILKKKDDNTYECSFYNPESTNSASDIE SKISMTGYSITNKNTSTSNESGVHFELTK
76.	MNDLKQFLYIALVCGVIAGLGAFLHIPQYPSMTIPRIVAILGIISAMLTFKDKQISASLK FSALLINVLPLCGTFVASN
77.	VSREMSYHWFKKMLLSTSILILSSSSLGLATHTVEAKDNLNGEKPTTNLNHNITSPSVNS EMNNNETGTPHESNQTGNEGTGSNSRDANPDSNNVKPDSNNQNPSTDSKPDPNNQNPSPN PKPDPDNPKPKPDPKPDPDKPKPNPDPKPDPDNPKPNPDPKPDPDPKPKPNPDPKPDPDKP KPNPNPKPDPNKPNPNPSPDPDQPGDSNHSGGSKNGGTWNPNASDGSNQGQWQPNGNQGN SQNPTGNDFVSQRFLALANGAYKYNPYILNQINKLGKDYGEVTDEDIYNIIRKQNFSGNA YLNGLQQQSNYFRFQYFNPLKSERYYRNLDEQVLALITGEIGSMPDLKKPEDKPDSKQRS FEPHEKDDFTVVKKQEDNKKSASTAYSKSWLAIVCSMMVVFSIMLFLFVKRNKKKNKNES QRR
78.	MKNKKRVLIASSLSCAILLLSAATTQANSAHKDSQDQNKKEHVDKSQQKDKRNVTNKDKN STAPDDIGKNGKITKRTETVYDEKTNILQNLQFDFIDDPTYDKNVLLVKKQGSIHSNLKF ESHKEEKNSNWLKYPSEYHVDFQVKRNRKTEILDQLPKNKISTAKVDSTFSYSSGGKFDS TKGIGRTSSNSYSKTISYNQQNYDTIASGKNNNWHVHWSVIANDLKYGGEVKNRNDELLF YRNTRIATVENPELSFASKYRYPALVRSGFNPEFLTYLSNEKSNEKTQFEVTYTRNQDIL KNRPGIHYAPPILEKNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYKEG
79.	MYTRTATTSDSQKNITQSLQFNFLFEPNYDKETVFIKAKGTIGSGLRILDPNGYWNSTLR WPGSYSVSIQNVDDNNNTNVTDFAPKNQDESREVKYTYGYKTGGDFSINRGGLTGNITKE SNYSETISYQQPSYRTLLDQSTSHKGVGWKVEAHLINNMGHDHTRQLTNDSDNRTKSEIF SLTRNGNLWAKDNFTPKDKMPVTVSEGFNPEFLAVMSHDKKDKGKSQFVVHYKRSMDEFK IDWNRHGFWGYWSGENHVDKKEEKLSALYEVDWKTHNVKFVKVLNDNEKK
80.	VVKFMNYPNGKPYRKNSAIDGGKKTAAFSNIEYGGRGMSLEKDIEHSNTFYLKSDIAVIH KKPTPVQIVNVNYPKRSKAVINEAYFRTPSTTDYNGVYQGYYIDFEAKETKNKTSFPLNN IHDHQVEHMKNAYQQKGIVFLMIRFKTLDEVYLLPYSKFEVFWKRYKDNIKKSITVDEIR KNGYHIPYQYQPRLDYLKAVDKLILDESEDRV
81.	VNTTKAALHGDVKLQNDKDHAKQTVSQLAHLNNAQKHMEDTLIDSETTRTAVKQDLTEAQ ALDQLMDALQQSIADKDATRASSAYVNAEPNKKQSYDEAVQNAESIIAGLNNPTINKGNV SSATQAVISSKNALDGVERLAQDKQTAGNSLNHLDQLTPAQQQALENQINNATTRGEVAQ KLTEAQALNQAMEALRNSIQDQQQTEAGSKFINEDKPQKDAYQAAVQNAKDLINQTNNPT LDKAQVEQLTQAVNQAKDNLHGDQKLADDKQHAVTDLNQLNGLNNPQRQALESQINNAAT RGEVAQKLAEAKALDQAMQALRNSIQDQQQTESGSKFINEDKPQKDAYQAAVQNAKDLIN QTGNPTLDKSQVEQLTQAVTTAKDNLHGDQKLARDQQQAVTTVNALPNLNHAQQQALTDA INAAPTRTEVAQHVQTATELDHAMETLKNKVDQVNTDKAQPNYTEASTDKKEAVDQALQA AESITDPTNGSNANKDAVDQVLTKLQEKENELNGNERVAEAKTQAKQTIDQLTHLNADQI ATAKQNIDQATKLQPIAELVDQATQLNQSMDQLQQAVNEHANVEQTVDYTQADSDKQNAY KQAIADAENVLKQNANKQQVDQALQNILNAKQALNGDERVALAKTNGKHDIDQLNALNNA QQDGFKGRIDQSNDLNQIQQIVDEAKALNRAMDQLSQEITDNEGRTKGSTNYVNADTQVK QVYDETVDKAKQALDKSTGQNLTAKQVIKLNDAVTAAKKALNGERRLNNRKAEALQRLDQ LTHLNNAQRQLAIQQINNAETLNKASRAINRATKLDNAMGAVQQYIDEQHLGVISSTNYI NADDNLKANYDNAIANAAHELDKVQGNAIAKAEAEQLKQNIIDAQNALNGDQNLANAKDK ANAFVNSLNGLNQQQQDLAHKAINNADTVSDVTDIVNNQIDLNDAMETLKHLVDNEIPNA EQTVNYQNADDNAKTNFDDAKRLANTLLNSDNTNVNDINGAIQAVNDAIHNLNGDQRLQD AKDKAIQSINQALANKLKEIEASNATDQDKLIAKNKAEELANSIINNINKATSNQAVSQV QTAGNHAIEQVHANEIPKAKIDANKDVDKQVQALIDEIDRNPNLTDKEKQALKDRINQIL QQGHNGINNAMTKEEIEQAKAQLAQALQDIKDLVKAKEDAKQDVDKQVQALIDEIDQNPN LTDKEKQALKDRINQILQQGHXDIXNAMTKEAIEQAKERLAQALQDIKDLVKAKEDAKND IDKRVQALIDEIDQNPNLTDKEKQALKDRINQILQQGHNDINNALTKEEIEQAKAQLAQA LQDIKDLVKAKEDAKNAIKALANAKRDQINSNPDLTPEQKAKALKEIDEAEKRALQNVEN AQTIDQLNRGLNLGLDDIRNTHVWEVDEQPAVNEIFEATPEQILVNGELIVHRDDIITEQ DILAHINLIDQLSAEVIDTPSTATISDSLTAKVEVTLLDGSKVIVNVPVKVVEKELSVVK QQAIESIENAAQQKINEINNSVTLTLEQKEAAIAEVNKLKQQAIDHVNNAPDVHSVEEIQ QQEQAHIEQFNPEQFTIEQAKSNAIKSIEDAIQHMIDEIKARTDLTDKEKQEAIAKLNQL KEQAIQAIQRAQSIDEISEQLEQFKAQMKAANPTAKELAKRKQEAISRIKDFSNEKINSI RNSEIGTADEKQAAMNQINEIVLETIRDINNAHTLQQVEAALNNGIARISAVQIVTSDRA KQSSSTGNESNSHLTIGYGTANHPFNSSTIGHKKKLDEDDDIDPLHMRHFSNNFGNVIKN AIGVVGISGLLASFWFFIAKRRRKEDEEEKLEIRDNNKDSIKETLDDTKHLPLLFAKRRR KEDEEDVTVEEKDSLNNGESLDKVKHTPFFLPKRRRKEDEEDVEVTNENTDEKVLKDNEH SPLLFAKRRKDKEEDVETTTSIESKDEDVPLLLAKKKNQKDNQSKDKKSASKNTSKKVAA KKKKKKAKKNKK

82.	MNQEVKNKIFSILKITPATALFIFVAITLYRELSGINFKDTLVRFSKINRMSLVLLFIGG GASLVILSMYDVILSRALKMDISLGKVLRVSYIINALNAIVGPGGFIGAGVRAMVYKNYT HDKKKLVHFISLILISMLTGLSLLSLLIVFHVFDASLILDKITWVRWVLYVVSPFLPLFI IYSMVRPPDKNNRFVGLYCTLVSCVEWLAAAVVLYFCGVIVDAHVSFMSFIAIFIIAALS GLVSFIPGGFGAFDLVVLLGFKTLGVPEEKVLLMLLLYRFAYYFVPVIIALILSSFEFGT SAKKYIEGSKYFIPAKDVTSFLMSYQKDIIAKIPSLSLAILVFFTSMIFFVNNLTIVYDA LYDGNHLTYYILLAIHTSACLLLLLNVVGIYKQSRRAIIFAMISILLITVATFFTYASYI LITWLAIIFVLLIVAFRRARRLKRPVRMRNIVAMLLFSLFILYVNHIFIAGTLYALDIYT IEMHTSVLRYYFWLTILIIAIIIGMIAWLFDYQFSKVRISSKIEDCEEIINQYGGNYLSH LIYSGDKQFFTNENKTAFLMYRYKASSLVVLGDPLGDENAFDELLEAFYNYAEYLGYDVI FYQVTDQHMPLYHNFGNQFFKLGEEAIIDLTQFSTSGKKRRGFRATLNKFDELNISFEII EPPFSTEFINELQHVSDLWLDNRQEMHFSVGEFNEEYLSKAPIGVMRNEENEVIAFCSLM PTYFNDAISVDLIRWLPELDLPLMDGLYLHMLLWSKEQGYTKFNMGMATLSNVGQLHYSY LRERLAGRVFEHFNGLYRFQGLRRYKSKYNPNWEPRFLVYRKDNSLWESLSKVMRVIRHK
83.	MVALTLVGSAVTAHQVQAAETTQDQTTNKNVLDSNKVKATTEQAKAEVKNPTQNISGTQV YQDPAIVQPKTANNKTGNAQVSQKVDTAQVNGDTRANQSATTNNTQPVAKSTSTTAPKTN TNVTNAGYSLVDDEDDNSENQINPELIKSAAKPAALETQYKTAAPKAATTSAPKARTEAT PKVTTFSASAQPRSVAATPKTSLPKYKPQVNSSINDYIRKNNLKAPKIEEDYTSYFPKYA YRNGVGRPEGIVVHDTANDRSTINGEISYMKNNYQNAFVHAFVDGDRIIETAPTDYLSWG VGAVGNPRFINVEIVHTHDYASFARSMNNYADYAATQLQYYGLKPDSAEYDGNGTVWTHY AVSKYLGGTDHADPHGYLRSHNYSYDQLYDLINEKYLIKMGKVAPWGTQSTTTPTTPSKP TTPSKPSTGKLTVAANNGVAQIKPTNSGLYTTVYDKTGKATNEVQKTFAVSKTATLGNQK FYLVQDYNSGNKFGWVKEGDVVYNTAKSPVNVNQSYSIKPGTKLYTVPWGTSKQVAGSVS GSGNQTFKASKQQQIDKSIYLYGSVNGKSGWVSKAYLVDTAKPTPTPTPKPSTPTTNNKL TVSSLNGVAQINAKNNGLFTTVYDKTGKPTKEVQKTFAVTKEASLGGNKFYLVKDYNSPT LIGWVKQGDVIYNNAKSPVNVMQTYTVKPGTKLYSVPWGTYKQEAGAVSGTGNQTFKATK QQQIDKSIYLFGTVNGKSGWVSKAYLAVPAAPKKAVAQPKTAVKAYTVTKPQTTQTVSKI AQVKPNNTGIRASVYEKTAKNGAKYADRTFYVTKERAHGNSTYVLLNNTSHNIPLGWFNV KDLNVQNLGKEVKTTQKYTVNKSNNGLSMVPWGTKNQVILTGNNIAQGTFNATKQVSVGK DVYLYGTINNRTGWVNAKDLTAPTAVKPTTSAAKDYNYTYVIKNGNGYYYVTPNSDTAKY SLKAFNEQPFAVVKEQVINGQTWYYGKLSNGKLAWIKSTDLAKELIKYNQTGMALNQVAQ IQAGLQYKPQVQRVPGKWTGANFNDVKHAMDTKRLAQDPALKYQFLRLDQPQNISIDKIN QPLKGKGVLENQGAAFNKAAQMYGINEVYLISHALLETGNGTSQLARGADVVNNKVVTNS NTKYHNVFGIAAYDNDPLREGIKYAKQAGWDTVSKAIVGGAKFIGNSYVKAGQNTLYKMR WNPAHPGTHQYATDVDWANINAKIIKGYYDKIGEVGKYFDIPQYK
84.	MKGKFLKVSSLFVATLTTATLVSSPAANALSSKAMDNHPQQTQSSKQQTPKIQKGGNLKP LEQREHANVILPNNDRHQITDTTNGHYAPVTYIQVEAPTGTFIASGVVVGKDTLLTNKHV VDATHGDPHALKAFPSAINQDNYPNGGFTAEQITKYSDEGDLAIVKFSPNEQHKHIGEVV KPATMSNNADTQVNQNITVTGYPGDKPVATMWESKGKITYLKGEAMQYDLSTTGGNSGSP VFNEKNEVIGIHWXGVPNEFNGAVFINENVRNFLKQNIEDIHFATMTNLITQIILITLTI LITLTTQMNQITLTTLTTLIIQTMAIXIIQTIQMQLN
85.	MQKKVIAAIIGTSAISAVAATQANAATTHTVKPGESVWAISNKYGISIAKLKSLNNLTSN LIFPNQVLKVSGSSNSTSNSSRPSTNSGGGSYYTVQAGDSLSLIASKYGTTYQNIMRLNG LNNFFIYPGQKLKVSGTASSSNAASNSSRPSTNSGGGSYYTVQAGDSLSLIASKYGTTYQ KIMSLNGLNNFFIYPGQKLKVTGNASTNSGSATTTNRGYNTPVFSHQNLYTWGQCTYHVF NRRAEIGKGISTYWWNANNWDNAAAADGYTIDNRPTVGSIAQTDVGYYGHVMFVERVNND GSILVSEMNYSAAPGILTYRTVPAYQVNNYRYIH
86.	MNNKKTATNRKGMIPNRLNKFSIRKYSVGTASILVGTTLIPGLSGHEAKAAEHTNGELNQ SKNETTAPSENKTTKKVDSRQLKDNTQTATADQPKVTMSDSATVKETSSNMQSPQNATAN QSTTKTSNVTTNDKSSTTYSNETDKSNLTQAKDVSTTPKTTTIKPRTLNRMAVNTVAAPQ QGTNVNDKVHFSNIDIAIDKGHVNQTTGKTEFWATSSDVLKLKANYTIDDSVKEGDTFTF KYGQYFRPGSVRLPSQTQNLYNAQGNIIARGIYDSTTNTTTYTFTNYVDQYTNVRGSFEQ VAFAKRKNATTDKTAYKMEVTLGNDTYSEEIIVDYGNKKAQPLISSTNYINNEDLSRNMT AYVNQPKNTYTKQTFVTNLTGYKFNPNAKNFKIYEVTDQNQFVDSFTPDTSKLKDVTDQF DVIYSNDNKTATVDLMKGQTSSNKQYIIQQVAYPDNSSTDNGKIDYTLDTDKTKYSWSNS YSNVNGSSTANGDQKKYNLGDYVWEDTNKDGKQDANEKGIKGVYVILKDSNGKELDRTTT DENGKYQFTGLSNGTYSVEFSTPAGYTPTTANVGTDDAVDSDGLTTTGVIKDADNMTLDS GFYKTPKYSLGDYVWYDSNKDGKQDSTEKGIKGVKVTLQNEKGEVIGTTETDENGKYRFD NLDSGKYKVIFEKPAGLTQTGTNTTEDDKDADGGEVDVTITDHDDFTLDNGYYEEETSDS DSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDS DSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDS DSDSDSDSDSDSDSDSDSDSDSDSDSDNDSDSDSDSDSDAGKHTPAKPMSTV KDQHKTAKALPETGSENNNSNNGTLFGGLFAALGSLLLFGRRKKQNK
87.	MDINSEEYKQEVLIKDVVMLAARILLESGAEGTRVEDIMTRIAKKLGYSESNSFVTNTVI QFTLHSESFPRIFRITSRDTNLIKISQANKISRQITNNEISLAEAKTQLEKIYVAKRDSS LPFKGFAAAMIAMSFLYLQGGRLIDVLTAILAGSLGYLVTEILDRKLHAQFIPEFIGSLV IGIIAVIGHTLIPTGDLATIIIAAVMPIVPGVLITNAIQDLFGGHMLMFTTKSLEALVTA FGIGAGVGSVLILV
88.	VIAIMNVIIDERKENAMTFNKVLLSWIVILIITTSIYLFWQLGDINDVFNQSILINVRLP RLLEALLTGMILTVAGLIFQTVLNNALADSFTLGLASGATPGSGLALFLGLTTLWIPVFS ITFSLITLITVLVITSVLSQGYPVRILILSGLMIGALFNSLLYFLILLKPRKLNTIANYL FGGFGDAEYSNVSIIAITFIIALFGIFIILNQLKLLQLGELKSQSLGLNVQLITYIALCI ASMITAINVAYVGIIGFIGMVIPQLIRKWQWKQSLGRQLALNIVTGGQIMVMADFIGSHI LSPVQIPASIIIALIGIPVLFYMLISQSKRLH
89.	MKKLAFAITATSGAAAFLTHHDAQASTQHTVQSGESLWSIAQKYNTSVESIKQNNQLDNN LVFPGQVISVGGSDAQNTSNTSPQAGSASSHTVQAGESLNIIASRYGVSVDQLMAANNLR GYLIMPNQTLQIPNGGSGGTTPTATTGSNGNASSFNHQNLYTAGQCTWYVFDRRAQAGSP ISTYWSDAKYWAGNAANDGYQVNNTPSVGSIMQSTPGPYGHVAYVERVNGDGSILISEMN YTYGPYNMNYRTIPASEVSSYAFIH
90.	MPDSITIIDENKVIDVVLIAGRILLESGAETYRVEDTMNRIAHSYGLHNTYSFVSSTAII PSLNDRTSTRLIRVQERTTDLEKIALTNSLSRKISNKELTIDEAKSEFIHLQHASLQYSF LTNFFAAAIACGFFLFMFGGVASDCWIAVIAGGSAFLTFSFVQRYIQIKFFSEFVAAAVV ISIAATFTKLGIATNQDIITIASVMPLVPGILITNAIRDLLAGELLAGMSRGVEAALTAF AIGAGVAIVLLII

91.	MGFLSKILDGNNKEIKQLGKLADKVIALEEKTAILTDEEIRNKTKQFQTELADIDNVKKQ NDYLDKILPEAYALVREGSKRVFNMTPYKVQIMGGIAIHKGDIAEMRTGEGKTLTATMPT YLNALAGRGVHVITVNEYLSSVQSEEMAELYNPLGLTVGLNLNSKTTEEKREAYAQDITY STNNELGFDYLRDNMVNYSEDRVMRPLHFAIIDEVDSILIDEARTPLIISGEAEKSTSLY TQANVFAKMLKQDEDYKYDEKTKAVHLTEQGADKAERMFKVENLYDVQNVDVISHINTAL RAHVTLQRDVDYMVVDGEVLIVDQFTGRTMPGRRFSEGLHQAIEAKEGVQIQNESKTMAS ITFQNYFRMYNKLAGMTGTAKTEEEFRNIYNMTVTQIPTNKPVQRNDKSDLIYISQKGK FDAVVEDVVEKHKAGQPVLLGTVAVETSEYISNLLKKRGIRHDVLNAKNHEREAEIVAGA GQKGAVTIATNMAGRGTDIKLGEGVEELGGLAVIGTERHESRRIDDQLRGRSGRQGDKGD SRFYLSLQDELMIRFGSERLQKMMSRLGLDDSTPIESKMVSRAVESAQKRVEGNNFDARK RILEYDEVLRKQREIIYNERNSIIDEEDSSQVVDAMLRSTLQRSINYYINTADDEPEYQP FIDYINDIFLQEGDITEDDIKGKDAEDIPEVVWAKIEAAYQSQKDILEEQMNEFERMILL RSIDSHWTDHIDTMDQLRQGIHLRSYAQQNPLRDYQNEGHELFDIMMQNIEEDTCKPILK SVVQVEDNIEREKTTEFGEAKHVSAEDGKEKVKPKPIVKGDQVGRNDDCPCGSGKKFKNC HGK
92.	MRESMSNQNYDYNKNEDGSKKKMSTTAKVVSIATVLLLLGGLVFAIFAYVDHSNKAKERM LNEQKQEQKEKRQKENAEKEKKKQQEEKEQNELDSQANQYQQLPQQNQYQYVPPQQQAP TKQRPAKEENDDKASKDESKDKDDKASQDKSDDNQKKTDDNKQPAQPKPQPQQPTPKPNN NQQNNQSNQQAKPQAPQQNSQSTTNKQNNANDK
93.	MNMKKKEKHAIRKKSIGVASVLVGTLIGFGLLSSKEADASENSVTQSDSASNESKSNDSS SVSAAPKTDDTNVSDTKTSSNTNNGETSVAQNPAQQETTQSSSTNATTEETPVTGEATTT TTNQANTPATTQSSNTNAEELVNQTSNETTSNDTNTVSSVNSPQNSTNAENVSTTQDTST EATPSNNESAPQSTDASNKDVVNQAVNTSAPRMRAFSLAAVAADAPAAGTDITNQLTNVT VGIDSGTTVYPHQAGYVKLNYGFSVPNSAVKGDTFKITVPKELNLNGVTSTAKVPPIMAG DQVLANGVIDSDGNVIYTFTDYVNTKDDVKATLTMPAYIDPENVKKTGNVTLATGIGSTT ANKTVLVDYEKYGKFYNLSIKGTIDQIDKTNNTYRQTIYVNPSGDNVIAPVLTGNLKPNT DSNALIDQQNTSIKVYKVDNAADLSESYFVNPENFEDVTNSVNITFPNPNQYKVEFNTPD DQITTPYIVVVNGHIDPNSKGDLALRSTLYGYNSNIIWRSMSWDNEVAFNNGSGSGDGID KFVVPEQPDEPGEIEPIPEDSDSDPGSDSGSDSNSDSGSDSGSDSTSDSGSDSASDSDSA SDSDSASDSDSASDSDSASDSDSDNDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDSESDSDSESDSDSDSDSDSDSDSDSDSDSDSDSASD SDSGSDSDSSSDSDSESDSNSDSESGSNNNVVPPNSPKNGTNASNKNEAKDSKEPLPDTG SEDEANTSLIWGLLASIGSLLLFRRKKENKDKK
94.	MNSNHAKASVTESVDKKFVVPESGINKIIPAYDEFKNSPKVNVSNLTDNKNFVASEDKLN KIADSSAASKIVDKNFVVPESKLGNIVPEYKEINNRVNVATNNPASQQVDKHFVAKGPEV NRFITQNKVNHHFITTQTHYKKVITSYKSTHVHKHVNHAKDSINKHFIVKPSESPRYTHP SQSLIIKHHFAVPGYHAHKFVTPGHASIKINHFCVVPQINSFKVIPPYGHNSHRMHVPSF QNNTTATHQNAKVNKAYDYKYFYSYKVVKGVKKYFSFSQSNGYKIGKPSLNIKNVNYQYA VPSYSPTHYVPEFKGSLPAPRV
95.	LEHTIMKMRTIAKTSLALGLLTTGAITVTTQSVKAEKIQSTKVDKVPTLKAERLAMINIT AGANSATTQAANTRQERTPKLEKAPNTNEEKTSASKIEKISQPKQEEQRTLNISATPAPK QEQSQTTTESTTPKTKVTTPPSTNTPQPMQSTKSDTPQSPTIKQAQTDMTPKYEDLRAYY TKPSFEFEKQFGFMLKPWITTVRFMNVIPNRFIYKIALVGKDEKKYKDGFYDNIDVFIVLE DNKYQLKKYSVGGITKTNSKKVNHKVELSITKKDNQGMISRDVSEYMITKEEISLKELDF KLRKQLIEKHNLYGNMGSGTIVIKMKNGGKYTFELHKKLQEHRMAGTNIDNIEVNIK
96.	MTTIKTSNLGFPRLGRKREWKKAIESYWAKKISKEELDQTLTLDLHKENLLLQKYYHLDSI PVGDFSLYDHILDTSLLFNIIPERFQGRTIDDDLLPDIARGNKDHVASALIKWFNTNYHY IVPEWDNVEPKVSRNVLLDRFKYAQSLNVNAHPVIVGPITFVKLSKGGHQTFEEKVKTLL PLYKEVFESLIDAGAEYIQVDEPILVTDDSESYENITREAYDYFEKAGVAKKLVIQTYFE RAHLKFLSSLPVGGIGLDFVHDNGYNLKQIEAGDFDKSKTLYAGIIDGRNVWASDIRAKK VLIDKLLAHTNELVIQPSSSLLHVPVSLDDETLDTSVGEGLSFATEKLDELDALRRLFNQ NDSVKYDKLKARYERFQNQSFKNLDYDFESVRTSRQSPFAQRIEQQQKRLNLPDLPTTTI GSFPQSREVRKYRADWKNKRITDEAYETFLKNEIARWIKIQEDIGLDVLVHGEFERNDMV EFFGEKLQGFLVTKFGWVQSYGSRAVKPPIIYGDVKWTAPLTVDETVYAQSLTDKPVKGM LTGPVTILNWSFERVDLPRKVVQDQIALAINEEVLALEAAGIKVIQVDEPALREGLPLRS EYHEQYLKDAVLSFKLATSSVRDETQIHTHMCYSQFGQIIHAIHDLDADVISIETSRSHG DLIKDFEDINYDLGIGLGVYDIHSPRIPTKEEITTAINRSLQQIDRSLFWVNPDCGLKTR KEEEVKDALTVLVNAVKAKRQE
97.	MSDTYKSYLVAVLCFTVLAIVLMPFLYFTTAWSIAGFASIATFIFYKEYFYEE
98.	MLRGQEERKYSIRKYSIGVVSVLAATMFVVSSHEAQASEKTSTNAAAQKETLNQPGEQGN AITSHQMQSGKQLDDMHKENGKSGTVTEGKDTLQSSKHQSTQNSKTIRTQNDNQVKQDSE RQGSKQSHQNNATNNTERQNDQVQNTHHAERNGSQSTTSQSNDVDKSQPSIPAQKVIPNH DKAAPTSTTPPSNDKTAPKSTKAQDATTDKHPNQQDTHQPAHQIIDARQDDTVRQSEQKP QVGDLSKHIDGQNSPEKPTDKNTDNKQLIKDALQAPKTRSTTNAAADAKKVRPLKANQVQ PLNKYPVVFVHGFLGLVGDNAPALYPNYWGGNKFKVIEELRKQGYNVHQASVSAFGSNYD RAVELYYYIKGGRVDYGAAHAAKYGHERYGKTYKGIMPNWEPGKKVHLVGHSMGGQTIRL MEEFLRNGNKEEIAYHKAHGGEISPLFTGGHNNMVASITTLATPHNGSQAADKFGNTEAV RKIMFALNRFMGNKYSNIDLGLTQWGFKQLPNESYIDYIKRVSKSKIWTSDDNAAYDLTL DGSAKLNNMTSMNPNITYTTYTGVSSHTGPLGYENPDLGTFFLMATTSRIIGHDAREEWR KNDGVVPVISSLHPSNQPFVNVTNDEPATRRGIWQVKPIIQGWD
99.	MIHLIKGKMHHTVLCIHLNKGVALMNQYHSNAQQPSAWRFFVYSLVGILCFFIPFTINGN NTIFVDHVHLAIRSIIGPLMPYVALIMILIGTALPIVRRTFMTSITNLVITLFKVAGAMI GIMYVFKIGPSILFKANYGPFLFEKLMMPLSILIPVGAIALSLLVGYGLLEFVGVYMEPI MRPIFKTPGKSAVDAVASFVGSYSLGLLITNRVYKQGMYNKREATIIATGFSTVSATFMI IVAKTLGLMPHWNLYFWITLVITFVVTAITAWLPPISNESTEYYNGQEGEQRVAIEGSRL KTAYARAMKQNALTPSLVKNVWDNLKDGLEMTVGILPSILSIGFLGLIVANYTPFIDWLG YIFYPFIYIFPIADQALLAKASAISIVEMFLPSLLVTKAAMSTKPVVGVVSVSAIIFFSA LVPCILATEIKIPVWKLIIIWFLRVALSLLITIPVALLIFG
100.	MVIMKKTILLTMTTLTLFSMSPNSAQAYTNDSKTLREAKKAHPNAQFKVNKDTGAYTYTY DKNNTPNNNHQNQSRTNDNHQHANQRDLNNNQYHSSLSGQYTHINDAIDSHTPPQTSPSN PLTPAIPNVEDNDDELNNAFSKDNKGLITGIDLDELYDELQIAEFNDKAKTADGKPLALG NGKIIDQPLITSKNNLYTAGQCTWYVFDKRAKDGHTISTFWGDAKNWAGQASSNGFKVDR HPTRGSILQTVNGPFGHVAYVEKVNIDGSILISEMNWIGEYIVSSRTISASEVSSYNYIH

101.	MEVSSMKPYIQLVVFKQWLQYILLVTTIVIALVLIGIGYRVAHDNFKIPITIQDLDQTTA SKSFVNKIKQSDYVTIKKVDEDESYIEDDVTKKEAILSMQIPKGFSQKLKENRLKETIQL YGRDDFIGGIAVEIVSSSLYEQQIPNIIYEHLEDMKQHQSIDAINKSYHKHTPESKIKFV SLTKQAQHSISISLIFAVILFVSAVQVVLHYRLNQQAALQRLSQYHLSRFKLYSTYVMTH TILLLLVLLAVSLYLSQPLSLIFYLKSLLLILIYEIGIVFILFHIQTISHRLFMTFIYAL AMGIVYLIIFM
102.	MIEVTEMNFFDIHKIPNKGIPLSVQRKLWLRNFMQAFFVVFFVYMAMYLIRNNFKAAQPF LKEEIGLSTLELGYIGLAFSITYGLGKTLLGYFVDGRNTKRIISFLLILSAITVLIMGFV LSYFGSVMGLLIVLWGLNGVFQSVGGPASYSTISRWAPRTKRGRYLGFWNTSHNIGGAIA GGVALWGANVFFHGNVIGMFIPPSVIALLIGIATLFIGKDDPEELGWNRABEIWEEPVDK ENIDSQGMTKWEIFKKYILGNPVIWILCVSNVFVYIVRIGIDNWAPLYVSEHLHFSKGDA VNTIFYFEIGALVASLLWGYVSDLLKGRRAIVAIGCMFMITFVVLFYTNATSVMMVNISL FALGALIFGPQLLIGVSLTGFVPKNAISVANGMTGSFAYLFGDSMAKVGLAAIADPTRNG LNIFGYTLSGWTDVFIVFYVALFLGMILLGIVAFYERKKIRSLKI
103.	MTKKKNILKAIGIYSFIAMMFVIILYPLLWTFGISLNPGTNLYGAKMIPDNATFKNYAFL LFDDSSQYLTWYKNTLIVASANALFSVIFVTLTAYAFSRYRFVGRKYGLITFLILQMFPV LMAMVAIYILLNTIGLLDSLFGLTLVYIGGSIPMNAFLVKGYFDTIPKELDESAKIDGAG HMRIFLQIMLPLAKPILAVVALFNFMGPFMDFILPKILLRSPEKFTLAVGLFNFINDKYA NNFTVFAAGAIMIAVPIAIVFLFLQRYLVSGLTTGATKG
104.	MMENSTTEARNEATMHLDEMTVEEALITMNKEDQQVPLAVRKAIPQLTKVIKKTIAQYKK GGRLIYIGAGTSGRLGVLDAAECVPTFNTDPHEIIGIIAGGQHAMTMAVEGAEDHKKLAE EDLKNIDLTSKDVVIGIAASGKTPYVIGGLTFANTIGATTVSISCNKHAVISEIAQYPVE VKVGPEVLTGSTRLKSGTAQKLILNMISTITMVGVGKVYDNLMIDVKATNQKLIDRSVRI IQBICAITYDEAMALYQVSEHDVKVATVMGMCGISKEEATRRLLNNGDIVKRAIRDRQP
105.	LQYIIRYIMMTLQIHTGGINLKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHD EAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNF NKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNK EQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQ QNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQN AFYEILHLPNLTEEQRNGFIQSLKDDPSVSKELLAEAKKLNDAQAPKEEDNNKPGKEDNN KPGKEDNNKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDNKKPGKEDGNKPGK EDGNKPGKEDGNGVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELVVDKK QPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRREL
106.	MDKKSEKRGIKMTVQSAYIHIPFCVRICTYCDFNKYFIQNQPVDEYLDALITEMSTAKYR ILKTMYVGGGTPTALSINQLERLLKAIRDTFTITGEYTFEANPDELTKEKVQLLEKYGVK RISMGVQTFKPELLSVLGRTHNTEDIYTSVLNAKNAGIKSISLDLMYHLPKQTIEDFEQS LDLALDMDIQHISSYGLILEPKTQFYNMYRKGLLKLPNEDLGADMYQLLMSKIEQSPFHQ YEISNFALDGHESEHNKVYWFNEEYYGFGAGASGYVDGVRYTNINPVNHYIKAINKESKA ILVSNKPSLTERMEEEMFLGLRLNEGVSSSRFKKKFDQSIESVFGQTINNLKEKELIVEK NDVIALTNRGKVIGNEVFEAFLIND
107.	atgaatgtattagtaattggtgctggtggacgagaacatgcacttgcatataaacttaat caatcgaatctagttaaacaagtgtttgtcattccaggtaatgaggcaatgacacctata gctgaagtacacactgaaatttcagaacctgatcatcaagcgatactagattttgctaaa cggcaaaatgttgattgggtagttataggtccagaacagccgctaattgatggattagca gacattttacgagcgaatggtttcaaagtgtttggtccaaataagcaagcagctcaaatc gaaggctcaaaattatttgctaaaaagataatggaaaaatataatattccaactgctgat tataaagaagttgagcgaaaaaaggatgctttaacatatattgaaaactgtgaattgccc gttgttgtcaagaaagatgggttagctgctgggaaaggcgttattattgcagatactatt gaagcagccagaagtgctattgagattatgtatggtgatgaagaagaggtactgttgta tttgaaacgtttttagaaggtgaagagttctcgctaatgacatttgttaatggtgattta gcagtacctttcgactgtattgcacaagatcataaacgcgcatttgatcatgatgaagga ccaaatactggtggtatgggggcttattgtccagtaccacatattagtgacgatgtttta aaacttacaaatgaaacaattgcacaacccattgcaaaggcaatgcttaatgaaggttat caattcttcggtgtattatacattggtgctattttaactaaagatggtccaaaagtaata gaatttaatgcccgttttggtgatcctgaagctcaagtattattaagtcgcatggaaagt gatttaatgcagcatattattgatttagatgaaggaaaacgtactgaattcaaatggaaa aatgaatctattgtaggggtcatgttggcatcaaaaggatatcctgatgcatatgaaaaa gggcataaagtaagtggctttgatttaaatgaaaactattttgttagtggattaaagaag caaggtgatacctttgttacttcaggtggtagagttatacttgccatcggaaaaggtgac aatgtacaagatgcacagcgagacgcatacaaaaaagtatcacaaatacaaagtgaccat ttattctatcgtcatgacattgcgaataaagcactacaacttaaa
108.	MNVLVIGAGGREHALAYKLNQSNLVKQVFVIPGNEAMTPIAEVHTEISEPDHQAILDFAK RQNVDWVVIGPEQPLIDGLADILRANGFKVFGPNKQAAQIEGSKLFAKKIMEKYNIPTAD YREVERKKDALTYIENCELPVVVKKDGLAAGKGVIIADTIEAARSAIEIMYGDEEEGTVV FETFLEGEEFSLMTFVNGDLAVPFDCIAQDHKRAFDHDEGPNTGGMGAYCPVPHISDDVL KLTNETIAQPIAKAMLNEGYQFPGVLYIGAILTKDGPKVIEFNARFGDPEAQVLLSRMES DLMQHIIDLDEGKRTEFKWKNESIVGVMLASKGYPDAYEKGHKVSGFDLNENYFVSGLKK QGDTFVTSGGRVILAIGKGDNVQDAQRDAYKKVSQIQSDHLFYRHDIANKALQLK
109.	atgcaaccacatttaatatgtctagacttagacggaacattattaaacgataacaaagaa atttcatcatatactaaacaagtattaaatgaattacaacaacgtggacaccaaattatg attgcgactggcagacctatcgtgcaagtcaaatgtattatcatgaattaaatttaacg acaccaattgttaattttaatggcgcttacgtacatcaccctaaagataaaaacttcaaa acttgccatgaaatttagatttaggcatcgcacaaaacattattcaaggattacaacaa tatcaagtatcgaatattatagcagaagtgaaagattatgttttcattaacaatcatgat ccaagattatttgaaggtttttcaatgggtaatccaagaattcaaactggtaatttactt gtccacttgaaagaatcccctacctcaattttaattgaagccgaagaaagtaaaatacct gaaatcaaaaatatgcttactcatttttatgccgatcatattgagcatcgacgctggggc gcaccattccctgtcattgaaattgtaaaacttggtattaataaagcaagaggcattgag caagttagacaatttttaaatattgaccgaaataatattattgcattcggtgatgaagat aatgatattgaaatgattgagtacgcgcgtcacggtgttgctatggaaaatggtttgcaa gaacttaaagatgtagcgaacaatattacattcaacaataatgaagatggcattggtcga tatttgaatgatttctttaatttaaatattagatattactgt
110.	MQPHLICLDLDGTLLNDNKEISSYTKQVLNELQQRGHQIMIATGRPYRASQMYYHRLNLT TPIVNFNGAYVHHPKDKNFKTCHEILDLGIAQNIIQGLQQYQVSNIIAEVKDYVFINNHD PRLFEGFSMGNPRIQTGNLLVHLKESPTSILIEAEESKIPEIKNMLTHFYADHIRHRRWG APFPVIEIVKLGINKARGIEQVRQFLNIDRNNIIAFGDEDNDIEMIEYARHGVAMENGLQ ELKDVANNITFNNNEDGIGRYLNDFFNLNIRYYC

111.

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aac

112.

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113.

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114.

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115.

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116.

MAKEKFDRSKEHANIGTIGHVDHGKTTLTAAIATVLAKNGDSVAQSYDMIDNAPEEKERG
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GDAQYEEKILELMEAVDTYIPTPERDSDKPFMMPVEDVFSITGRGTVATGRVERGQIKVG
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117.	atgactaagagtgctttagtaacaggtgcatcaagaggaattggacgtagtattgcgtta caattagcagaagaaggatataatgtagcagtaaactatgcaggcagcaaagagaaagct gaagcagtagtcgaagaaatcaaagctaaaggtgttgaaagttttgcgattcaagcaaat gttgccgatgcatgaaaatgactaagagtgctttagtaacaggtgcatcaagaggaattg gacgtagtattgcgttacaattagcagaagaaggatataatgtagcagtaaactatgcag gcagcaaagagaaagctgaagcagtagtcgaagaaatcaaagctaaaggtgttgaaagtt ttgcgattcaagcaaatgttgccgatgctgatgaagttaaagcaatgattaaagaagtag ttagccaatttggttctttagatgttttagtaaataatgcaggtattactcgcgataatt tattaatgcgtatgaaagaacaagagtgggatgatgttattgacacaaacttaaaaggtg tatttaactgtatccaaaaagcaacaccacaaatgttaagacaacgtagtggtgctatca tcaatttatcaagtgttgttggagcagtaggtaatccgggacaagcaaactatgttgcaa caaaagcaggtgttattggtttaactaaatctgcggcgcgtgaattagcatctcgtggta tcactgtaaatgcagttgcacctggttttattgtttctgatatgacagatgctttaagtg atgagcttaaagaacaaatgttgactcaaattccgttagcacgttttggtcaagacacag atattgctaatacagtagcgttcttagcatcagacaaagcaaaatatattacaggtcaaa caatccatgtaaatggtggaatgtacatg
118.	MTKSALVTGASRGIGRSIALQLAEEGYNVAVNYAGSKEKAEAVVEEIKARGVESFAIQAN VADADEVKAMIKEVVSQFGSLDVLVNNAGITRDNLLMRMKEQEWDDVIDTNLKGVFNCIQ KATPQMLRQRSGAIINLSSVVGAVGNPGQANYVATKAGVIGLTKSAARELASRGITVNAV APGFIVSDMTDALSDELKEQMLTQIPLARFGQDTDIANTVAFLASDKAKYITGQTIHVNG GMYM
119.	atgaaaatttctactaaagggagatatggacttacattgatgatttctcttgctaaaaaa gaggggcaaggatgtatatcattaaagtcaattgctgaagaaaataatttgagtgattta tatttagaacagcttgtaggtcctttaagaaatgcggggttaattcgaagtgtacgcggt gctaaaggtggataccaattaagagtgccagcggaagaaatctcagcaggggatattata agactgttagaaggtccaattacatttgttgaaagtattgaatcagaaccacctgcgcaa aaacaactatggattcgcatgagagatgcagtgagagatgttttagataatacaacattg aaatatttagcggaatatgtagatacaagtgaagatttagacggatacatgttttatatt
120.	MLKISTKGRYGLTIMIELAKKHGEGPTSLKSIAQTNNLSEHYLEQLVSPLRNAGLVKSIR GAYGGYVLGSEPDAITAGDIIRVLEGPISLLKCWKMRSLPSVSSGFASGML
121.	gtggcatttgaatttagattacccgatatcggggaaggtatccacgaaggtgaaattgta aaatggtttgttaaagctggagatactattgaagaagacgatgttttagctgaggtacaa aacgataaatcagtagtagaaatcccatcaccagtatctggtactgtagaagaagttatg gtagaagaaggtacagtagctgtagttggtgacgttattgttaaaatcgatgcacctgat gcagaagatatgcaatttaaaggtcatgatgatgattcatcatctaaagaagaacctgcg aaagaggaagcgccagcagagcaagcacctgtagctactcaaactgaagaagtagatgaa aacagaactgttaaagcaatgccttcagtacgtaaatacgcacgtgaaaaaggtgttaac attaaagcagtttctggatctggtaaaaatggtcgtattacaaaagaagatgtagatgca tacttaaatggtggtgcaccaacagcttcaaatgaatcagctgcttcagctacaagtgaa gaagttgctgaaactcctgcagcacctgcagcagtaacattagaaggcgacttcccagaa acaactgaaaaaatccctgctatgcgtagagcaattgcgaaagcaatggttaactctaag catactgcacctcatgtaacattaatggatgaaattgatgttcaagcattatgggatcac cgtaagaaatttaaagaaatcgcagctgaacaaggtactaagttaacattcttaccttat gttgttaaagcacttgtttctgcattgaaaaaatacccagcacttaacacttcattcaat gaagaagctggtgaaatcgttcataaacattactggaatatcggtattgcagcagacact gatagaggattattagtacctgttgttaaacatgctgatcgtaagtctattttccaaatt tcagatgaaattaatgaattagctgttaaagcacgtgatggtaaattaacagccgatgaa atgaaaggtgctacatgcacaatcagtaatatcggttcagctggtggacaatggttcact ccagttatcaatcacccagaagtagcaatcttaggaattggccgtattgctcaaaaacct atcgttaaagatggagaaattgttgcagcaccagtattagcattatcattaagctttgac cacagacaaattgatggtgcaactggccaaaatgcaatgaatcacattaaacgtttatta aataatccagaattattattaatggagggg
122.	MAFEFRLPDIGEGIHEGEIVKWFVKAGDTIEEDDVLAEVQNDKSVVEIPSPVSGTVEEVM VEEGTVAVVGDVIVKIDAPDAEDMQFKGHDDDSSSKEEPAKEEAPAEQAPVATQTEEVDE NRTVKAMPSVRKYAREKGVNIKAVSGSGKNGRITKEDVDAYLNGGAPTASNESAASATSE EVAETPAAPAAVTLEGDFPETTEKIPAMRRAIAKAMVNSKHTAPHVTLMDEIDVQALWDH RKKFKEIAAEQGTKLTFLPYVVKALVSALKKYPAINTSFNEEAGEIVHKHYWNIGIAADT DRGLLVPVVKHADRKSIFQISDEINELAVKARDGKLTADEMKGATCTISNIGSAGGQWFT PVINHPEVAILGIGRIAQKPIVKDGEIVAAPVLALSLSFDHRQIDGATGQNAMNHIKRLL

123.

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124.

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125.

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126.
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127.
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128.
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129.
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130.
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131.
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132.
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133.	gtggaattacaattagcaattgatttattaaacaaagaagacgcggctgagttagcaaat aaagtaaaagattatgtagatatcgtagaaatcggtacgccaatcatttacaacgaaggt ttaccagcagttaaacatatggcagacaacattagtaatgtaaaagtattagcagacatg aaaattatggatgcagctgattatgaagttagccaagcaattaaatttggcgcggatgta attacaatactaggtgttgcagaagatgcatcaattaaagcagctattgaagaagctcat aaaaataataaacaattactagttgatatgattgctgttcaagatttagaaaaacgtgca aaagaactagatgaaatgggtgctgattatattgcagtacacactggttatgatttacaa gcagaagggcaatcaccattagaaagtttaagaaccgttaaatctgttattaaaaattct aaagttgcagtagcaggtggaattaaaccagatacaattaaagatattgtcgctgaaagt cctgatcttgttattgttggtggcggaatcgcaaatgcagatgatccagtagaagctgca aaacaatgtcgcgctgcaatcgaaggtaag
134.	MELQLAIDLLNKEDAAELANKVKDYVDIVEIGTPIIYNEGLPAVKHMADNISNVKVLADM KIMDAADYEVSQAIKPGADVITILGVAEDASIKAAIEEAHKNNKQLLVDMIAVQDLEKRA KELDEMGADYIAVHTGYDLQAEGQSPLESLRTVKSVIKNSKVAVAGGIKPDTIKDIVAES PDLVIVGGGIANADDPVEAAKQCRAAIEGK
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136.	MKKLVPLLLALLLLVAACGTGGKQSSDKSNGKLKVVTTNSILYDMAKNVGGDNVDIHSIV PVGQDPHEYEVKPKDIKKLTDADVILYNGLNLETGNGWFEKALEQAGKSLKDKKVIAVSK DVKPIYLNGEEGNKDKQDPHAWLSLDNGIKYVKTIQQTFIDNDKKHKADYEKQGNKYIAQ LEKLNNDSKDKFNDIPKEQRAMTSEGAFKYFSKQYGITPGYIWEINTEKQGTPEQMRQA IEFVKKHKLKHLLVETSVDKKAMESLSEETKKDIFGEVYTDSIGKEGTKGDSYYKMMKSN IETVHGSMK
137.	atgacaactgatattttgaacatttctgaagaacaacttgttgattattctaaagcccac aatgaaccttcttggatgacagaattacgtaaaaaagctttgaaattaacagaaacttta gaaatgccaaaacctgataaaacaaaattaagaaaatgggattttgattcttttaaacaa cacgatgtaaaaggtgatgtttatcaatctttatcacaattacctgagtcagtaagagaa attattgacgtagatcattctaaaaacttagtaattcaacataataatacgattgcgtac acacaagttgatgataatgcatcgaaagatggcgttatcgttgaaggtttagcagacgct cttatgaaccatagtgatttagtacaaaagtactttatgaaagatgcagtaacagtagat gaacatcgtatcacagcgctacacacggcattagttaatggtggcgtatttgtttatgtt cctaaaaatgtagttgtagaacatccagtacaatacgttgtgttgcacgacgacgaaaat gcaagcttttataaccatgttatcatcgttactgaagaaagcgccgaagtcacatatgtt gaaaattacttatcaaatgcatctggtgaaggaaatcaattaaatattatttctgaagtg attgctggtgcaaattcaaatatcacatatggctcagtggactatatggataaaggcttt acaggtcatatcattcgacgtggtattactgaagcggatgcctcaattaattgggcacta ggtttaatgaatgagggtagccaaattattgataatacaacaaatttatttggtgatcgt tcaacaagttcacttaaatcagtagttgtaggtacaggcgaacaaaaaattaatctaaca tctaaaatcgtacaatatggtaaagaaacagatggttatatccttaaacatggtgttatg aaagaacatgcatcgtctgtatttaatggtatcggctacattaagcatggtggaactaaa tcaattgctaatcaggaatcacgtgtattaatgttatctgaacatgctcgtggtgacgcg aatcctatttttattaattgatgaagatgatgtacaagctggtcatgctgcatcagtaggt cgtgttgatccagatcaactttactatttaatgagtcgtggtatttctcaaagagaagcg gaacgtcttgttatacatggtttcttagatccagtagtacgtgaattacctatcgaagac gttaaacgtcaattgagagaagtaattgaacgcaaagtttctaaa
138.	MTTDILNISEEQLVDYSKAHNEPSWMTELRKKALKLTETLEMPKPDKTKLRKWDFDSFKQ HDVKGDVYQSLSQLPESVREIIDVDHSKNLVIQHNNTIAYTQVDDNASKDGVIVEGLADA LMNHSDLVQKYFMKDAVTVDEHRITALHTALVNGGVFVYVPKNVVVEHPVQYVVLHDDEN ASFYNHVIIVTEESAEVTYVENYLSNASGEGNQLNIISEVIAGANSNITYGSVDYMDKGF TGHIIRRGITEADASINWALGLMNEGSQIIDNTTNLFGDRSTSSLKSVVVGTGEQKINLT SKIVQYGKETDGYILKHGVMKEHASSVFNGIGYIKHGGTKSIANQESRVLMLSEHARGDA NPILLIDEDDVQAGHAASVGRVDPDQLYYLMSRGISQREAERLVIHGFLDPVVRELPIED VKRQLREVIERKVSK

139.

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140.

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141.

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142.

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143.

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144.

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145.	atgattaacagggataataaaaaggcaataacaaaaaagggtatgatttcaaatcgctta aacaaattttcgattagaaagtatactgtaggaactgcatcgattttagtaggtacgaca ttgatttttggtctagggaaccaagaagctaaagctgctgaaaacactagtacagaaaat gcaaaacaagatgatgcaacgactagtgataataaagaagtagtgtcggaaactgaaaat aattcgacaacagaaaataattcaacaaatccaattaagaaagaaacaaatactgattca caaccagaagctaaaaaagaatcaacttcatcaagtactcaaaaacagcaaaataacgtt acagctacaactgaaactaagcctcaaaacattgaaaaagaaaatgttaaaccttcaact gataaaactgcgacagaagatacatctgttatttagaagagaagaaagcaccaaataat acaaataacgatgtaactacaaaaccatctacaagtgaaccatctacaagtgaaattcaa acaaaaccaactacacctcaagaatctacaaatattgaaaattcacaaccgcaaccaacg ccttcaaaagtagacaatcaagttacagatgcaactaatccaaaagaaccagtaaatgtg tcaaaagaagaacttaaaaaaaatcctgagaaattaaaagaattggttagaaatgatagc aatacagatcattcaactaaaccagttgctacagctccaacaagtgttgcaccaaaacgt gtaaacgcaaaaatgcgctttgcagttgcacaaccagcagcagttgcttcaaacaatgta aatgatttaattaaagtgacgaagcaaacaatcaaagttggcgatggtaaagataatgtg gcagcagcgcatgacggtaaagatattgaatatgatacagagtttacaattgacaataaa gtcaaaaaaggcgatacaatgacgattaattatgataagaatgtaattccttcggattta acagataaaaatgatcctatcgatattactgatccatcaggagaggtcattgctaaagga acatttgataaagcaactaagcaaatcacatatacatttacagactatgtagataaatat gaagatataaaatcacgcttaactctatattcgtatattgataaaaaaacagttccaaat gagacaagtttgaatttaacatttgctacagcaggtaaagaaacaagccaaaatgtcact gttgattatcaagatccaatggtccatggtgattcaaacattcaatctatctttacaaaa ttagatgaagataagcaaactattgaacaacaaatttatgttaacccattgaaaaaatca gcaaccaacactaaagttgatatagctggtagtcaagtagatgattatggaaatattaaa ctaggaaatggtagcaccattattgaccaaaatacagaaataaaggtttataaagttaac tctgatcaacaattgcctcaaagtaatagaatctatgattttagtcaatacgaagatgta acaagtcaatttgataataaaaaatcatttagtaatatgtagcaacattggattttggt gatattaattcagcctatattatcaaagttgttagtaaatatacacctacatcagatggc gaactagatattgcccaaggtactagtatgagaacaactgataaatatggttattataat tatgcaggatattcaaacttcatcgtaacttctaatgacactggcggtggcgacggtact gttaaacctgaagaaaagttatacaaaattggtgactatgtatgggaagacgttgataaa gacggtgttcaaggtacagattcaaaagaaaaaccaatggcaaacgttttagttacatta acttacccggacggtactacaaaatcagtaagaacagatgctaatggtcattatgaattc ggtggtttgaaagacggagaaacttatacagttaaattcgaaacgccaactggatatctt ccaacaaaagtaaatggaacaactgatggtgaaaaagactcaaatggtagttcggttact gttaaaattaatggtaaagatgatatgtctttagatactggtttttacaaagaacctaaa tacaacttaggtgactatgtatgggaagatactaataaagatggtatccaagatgcaaat gagccaggaatcaaagatgttaaggttacattaaaagatagtactggaaaagttattggt acaactactactgatgcctcgggtaaatataaatttacagatttagataatggtaactat acagtagaatttgaaacaccagcaggttacacgccaacggttaaaaatactacagctgat gataaagattctaatggtttaacaacaacaggtgtcattaaagatgcagataatatgaca ttagacaggggtttctataaaacaccaaaatacagtttaggtgattatgtttggtacgac agtaataaagacggcaaacaagattcaactgaaaaaggtatcaaagatgtgacagttaca ttgcaaaacgaaaaaggcgaagtaattggaacaactaaaacagatgaaaatggtaaatat cgtttcgataatttagatagcggtaaatacaaagttatttttgaaaagcctgctggctta acacaaacagttacaaatacaactgaagatgataaagatgcagatggtggcgaagttgac gtaacaattacggatcatgatgatttcacacttgataacggatacttcgaagaagatataca tcagacagcgattcagactcagatagtgactcagacagcgactcagactcagacagcgac tcagactcagacagtgattcagattcagacagcgactcagattcagatagcgactcagat tcggacagcgattcagactcagatagcgactcagattcagatagcgattcagactcagac agcgactcagattcagatagcgattcggactcagacagcgattcagactcagatagcgac tcagactcagacagcgactcagattcagatagcgattcggactcagatagcgactcagat tcagacagcgattcagactcagatagcgactcagattcagacagcgattcagactcagat agcgactcagactcagacagtgattcagattcagacagcgactcagactcagatagcgac tcagattcggacagcgactcagactcagatagcgactcagactcagacagtgattcagac agcgattcagactcggatgcaggaaaacatacacctgttaaaccaatgagtactactaaa gaccatcacaataaagcaaaagcattaccagaaacaggtagtgaaaataacggctcaaat aacgcaacgttatttggtggattatttgcagcattaggttcattattgttattcggtcgt cgcaaaaaacaaaacaaa
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148.

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149.

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150.

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151.

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152.	MPKRNDIKTILVIGSGPIIIGQAAEFDYAGTQACLALKEEGYRVILVNSNPATIMTDKEI ADKVYIEPLTHDFIARIIRKEQPDALLPTLGGQTGLNMAIQLHESGVLQDNNVQLLGTEL TSIQQAEDREMFRTLMNDLNVPVPESDIVNTVEQAFKFKEQVGYPLIVRPAFTMGGTGGG ICHNDEELHEIVSNGLHYSPATQCLLEKSIAGFKEIEYEVMRDKNDNAIVVCNMENIDPV GIHTGDSIVVAPSQTLSDVEYQMLRDVSLKVIRALGIEGGCNVQLALDPHSFDYYIIEVN PRVSRSSALASKATGYPIAKLAAKIAVGLTLDEMLNPITGTSYAAFEPTLDYVISKIPRF PFDKFEKGERELGTQMKATGEVMAIGRTYEESLLKAIRSLEYGVHHLGLPNGESFDLDYI KERISHQDDERLFFIGEAIRRGTTLREIHNMTQIDYFFLHKFQNIIDIEHQLKEHQGDLE YLKYAKDYGFSDKTIAHRFNMTEEEVYQLRMENDIKPVYKMVDTCAAEFESSTPYYYGTY ETENESIVTDKEKILVLGSGPIRIGQGVEFDYATVHAVWAIQKAGYEAIIVNNNPETVST DFSISDKLYFEPLTEEDVMNIINLEKPKGVVVQFGGQTAINLADKLAKHGVKILGTSLEN LNRAEDRKEFEALLRKINVPQPQGKTATSPEEALANAAEIGYPVVVRPSYVLGGRAMEIV DNDKELENYMTQAVKASPEHPVLVDRYLTGKEIEVDAICDGETVIIPGIMEHIERAGVHS GDSIAVYPPQTLTEDELATLEDYTIKLAKGLNIIGLINIQFVIAHDGVYVLEVNPRSSRT VPFLSKITDIPMAQLAMRAIIGEKLTDMGYQEGVQPYAEGVFVKAPVFSFNKLKNVDITL GPEMKSTGEVMGKDTTLEKALFKGLTGSGVEVKDHGTVLMTVSDKDKEEVVKLAQRLNEV GYKILATSGTANKLAEYDIPAEVVGKIGGENDLLTRIQNGDVQIVINIMTKGKEVERDGF QIRRTTVENGIPCLTSLDTANALTNVIESMTFTMRQM
153.	MINRDNKKAITKKGMISNRLNKFSIRKYTVGTASILVGTTLIFGLGNQEAKAAENTSTEN AKQDDATTSDNKEVVSETENNSTTENNSTNPIKKETNTDSQPEAKKESTSSSTQKQQNNV TATTETKPQNIEKENVKPSTDKTATEDTSVILEEKKAPNNTNNDVTTKPSTSEPSTSRIQ TKPTTPQESTNIENSQPQPTPSKVDNQVTDATNPKEPVNVSKEELKKNPEKLKELVRNDS NTDHSTKPVATAPTSVAPKRVNAKMRFAVAQPAAVASNNVNDLIKVTKQTIKVGDGKDNV AAAHDGKDIEYDTEFTIDNKVKKGDTMTINYDKNVIPSDLTDKNDPIDITDPSGEVIAKG TFDKATKQITYTFTDYVDKYEDIKSRLTLYSYIDKKTVPNETSLNLTFATAGKETSQNVT VDYQDPMVHGDSNIQSIFTKLDEDKQTIEQQIYVNPLKKSATNTKVDIAGSQVDDYGNIK LGNGSTIIDQNTEIKVYKVNSDQQLPQSNRIYDFSQYEDVTSQFDNKKSFSNNVATLDFG DINSAYIIKVVSKYTPTSDGELDIAQGTSMRTTDKYGYYNYAGYSNFIVTSNDTGGGDGT VKPEEKLYKIGDYVWEDVDKDGVQGTDSKEKPMANVLVTLTYPDGTTKSVRTDANGHYEF GGLKDGETYTVKFETPTGYLPTKVNGTTDGEKDSNGSSVTVKINGKDDMSLDTGFYKEPK YNLGDYVWEDTNKDGIQDANEPGIKDVKVTLKDSTGKVIGTTTTDASGKYKFTDLDNGNY TVEFETPAGYTPTVKNTTADDKDSNGLTTTGVIKDADNMTLDRGFYKTPKYSLGDYVWYD SNKDGKQDSTEKGIKDVTVTLQNEKGEVIGTTKTDENGKYRFDNLDSGKYKVIFEKPAGL TQTVTNTTEDDKDADGGEVDVTITDHDDFTLDNGYFEEDTSDSDSDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSDSD SDSDSDSDSDSDSDSDSDSDSDSDAGKHTPVKPMSTTKDHHNKAKALPETGSENNGSN NATLFGGLFAALGSLLLFGRRKKQNK
154.	MTHLLETFEMSIDHQEDGLVVISMPVTDKVKQPFGYLHGGASIALGETACSLGSANLIDT TKFIPLGLEMNANHIHSAKDGRVTATAEIIHRGKSTHVWDIKIKNDKEQLITVMRGTVAI KPLK
155.	MEHTTMKITTIAATSLALGLLTTGVITTTTQAANATTPSSTKVEAPQSTPPSTKIEAPQS KPNATTPPSTKVEAPQQTANATTPPSTKVTTPPSTNTPQPMQSTKSDTPQSPTTKQVPTE INPKFKDLRAYYTKPSLEFKNEIGIILKKWTTIRFMNVVPDYFIYKIALVGKDDKKYGEG VHRNVDVFVVLEENNYNLEKYSVGGITKSNSKKVDHKAGVRITKEDNKGTISHDVSEFKI TKEQISLKELDFKLRKQLIEKNNLYGNVGSGKIVIKMKNGGKYTFELHKKLQENRMADVI NSEQIKNIEVNLK
156.	MKKQIISLGALAVASSLFTWDNKADAIVTKDYSGKSQVNAGSKNGTLIDSRYLNSALYYL EDYIIYAIGLTNKYEYGDNIYKEAKDRLLEKVLREDQYLLERKKSQYEDYKQWYANYKKE NPRTDLKMANFHKYNLEELSMKEYNELQDALKRALDDFHREVKDIKDKNSDLKTFNAAEE DKATKEVYDLVSEIDTLVVSYYGDKDYGEHAKELRAKLDLILGDTDNPHKITNERIKKEM IDDLNSIIDDFFMETKQNRPKSITKYNPTTHNYKTNSDNKPNFDKLVEETKKAVKEADDS WKKKTVKKYGETETKSPVVKEEKKVEEPQAPKVDNQQEVKTTAGKAEETTQPVAQPLVKI PQGTITGEIVKGPEYPTMENKTVQGEIVQGPDFLTMEQSGPSLSNNYTNPPLTNPILEGL EGSSSKLEIKPQGTESTLKGTQGESSDIEVKPQATETTEASQYGPRPQFNKTPKYVKYRD AGTGIREYNDGTFGYEARPRFNKPSETNAYNVTTHANGQVSYGARPTYKKPSETNAYNVT THANGQVSYGARPTQNKPSKTNAYNVTTHGNGQVSYGARPTQNKPSKTNAYNVTTHANGQ VSYGARPTYKKPSKTNAYNVTTHADGTATYGPRVTK
157.	MKKLATVGSLIVTSTLVFSSMPFQNAHADTTSMNVSNKQSQNVQNHRPYGGVVPQGMTQA QYTELEKALPQLSAGSNMQDYNMKLYDATQNIADKYNVIITTNVGVFKPHAVRDMNGHAL PLTKDGNFYQTNVDANGVNHGGSEMVQNKTGHMSQQGHMNQNTHMNQQPHMQQGHMQSSN HQMMSPKANMHSSNHQMNQSNKKVLPAAGESMTSSILTASIAALLLVSGLFLAFRRRRSTNK
158.	VLRSDFYMSYSIVRVSKVKSGTNTTGIQKHVQRENNNYENEDIDHSKTYLNYDLVNANKQ NFNNLIDEKIEQNYTGKRKIRTDAIKHIDGLITSDNDFFDNQTPEDTKQFFEYAKEFLEQ EYGKDNLLYATVHMDEKTPHMHYGVVPITDDGRLSAKEVVGNKKALTAFQDRFNEHVKQR GYDLERGQSRQVTNAKHEQISQYKQKTEYHKQEYERESQKTDHIKQNDKLMQEYQKSLN TLKKPINVPYEQETEKVGGLFSKEIQETGNVVISQKDFNEFQKQIKAAQDISEDYEYIKS GRALDDKDKEIREKDDLLNKAVERIENADDNFNQLYENAKPLKENIEIALKLLKILLKEL ERVLGRNTFAERVNKLTEDEPMA

159.

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160.

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161.

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162.	MNSEFIYGRVTNLGGRILSLIKKKNKDIRIIPLGGVGEIAKNMYIVEVDDEMFMLDAGLM FPEDEMLGIDIVIPDISYVLENKDKLKGIFLTHGHEHAIGAVSYVLEQLDAPVYGSKLTI ALIKENMKARNIDKKVRYYTVNNDSIMRPKNVNISFFNTTHSIPDSLGVCIHTSYGAIVY TGEFKFDQSLHGHYAPDIKRMAEIGEEGVFVLISDSTEAEKPGYNTPENVIEHHMYDAFA KVRGRLIVSCYASNFIRIQQVLNIASKLNRKVSFLGRSLESSFNIARKMGYFDIPKDLLI PITEVDNYPKNEVIIIATGMQGEPVEALSQMAQHKHKIMNIEEGDSVFLAITASANMEVI IANTLNELVRAGAHIIPNNKKIHASSHGCMEELKMMINIMKPEYFIPVQGEFKMQIAHAK LAAEAGVAPEKIFLVEKGDVINYNGKDMILNEKVNSGNILIDGIGIGDVGNIVLRDRHLL AEDGIFIAVVTLDPKNRRIAAGPEIQSRGFVYVRESEDLLREAEEKVREIVEAGLQEKRI EWSEIKQNMRDQISKLLFESTKRRPMIIPVISEI
163.	atggaaataacaatgcctaagttaggtgagagtgttcatgaaggcaccattgaacaatgg ttagtttctgttggtgatcatattgatgaatatgaaccattatgtgaagttattacagat aaagtgacagctgaagtcccttccacgatatcaggaacaattacagaaattttagttgaa gcggggcagacagtagctattgatacaattatctgtaaaattgaaactgctgatgaaaag acaaatgaaacaactgaagagatacaagcaaaagtggatgagcatactcagaaatctact aaaaaagctagtgcaacagtggaacagacatctactgctaaacaaaatcaaccacgtaat aatggtcgcttttcacctgttgtatttaaactcgcttcagagcatgacattgatttatca caagttgtaggtagtggatttgaaggtcgtgtaactaagaaggatataatgtcagttatt gaaaatggtggtaccacagctcaatctgacaaacaagttcaaacaaaatcaacatcagta gatacatcaagtaaccaatcatctgaagacaatagtgaaaacagcacaataccagtaaat ggtgtgcgtaaagcaattgcgcaaaatatggttaatagtgtaacagagattccacatgca tggatgatgattgaagtagatgctacaaatcttgtgaaaacgagaaatcattataaaaac agctttaaaaataaagaaggatataatctaacgttctttgctttctttgtaaaagctgta gcagatgctttaaaagcatatcctttattaaatagtagctggcaaggaaatgaaattgtc ttacataaagacattaatatttcaattgctgttgctgatgaaaataaattatacgtacct gtgattaagcatgcagacgaaaagtcaatcaaaggtatagctagagaaattaatacttta gcaacgaaagcgcgtaataagcaattgacagctgaagatatgcagggcggtacatttacg gtaaataatactggtacatttggttcagtatcatcaatgggtattataaatcatccacaa gcagcgattttacaagtagaatcaatcgttaaaaagccagtagtaattaatgatatgatt gcaattcgtaacatggttaatttatgtatttcaattgatcatcgtattttagatggttta caaacaggtaaatttatgaatcatattaaacagcgtatcgaacagtatactttagaaaat acaaatatatat
164.	MEITMPKLGESVHEGTIEQWLVSVGDHIDEYEPLCEVITDKVTAEVPSTISGTITEILVE AGQTVAIDTIICKIETADEKTNETTEEIQAKVDEHTQKSTKKASATVEQTSTAKQNQFRN NGRFSPVVFKLASEHDIDLSQVVGSGFEGRVTKKDIMSVIENGGTTAQSDKQVQTKSTSV DTSSNQSSEDNSENSTIPVNGVRKAIAQNMVNSVTEIPHAWMMIEVDATNLVKTRNHYKN SFKNKEGYNLTFFAFFVKAVADALKAYPLLNSSWQGNEIVLHKDINISIAVADENKLYVP VIKHADEKSIKGIAREINTLATKARNKQLTAEDMQGGTFTVNNTGTFGSVSSMGIINHPQ AAILQVESIVKKPVVINDMIAIRNMVNLCISIDHRILDGLQTGKFMNHIKQRIEQYTLEN TNIY
165.	
166.	
167.	atggaggacaacatgatttatgcaggtattttagcaggaggtattggttcgagaatgggg aacgtgccattaccaaaacaatttttagatattgataataaaccgattttaatccataca attgagaagttcattttagtgagtgaatttaatgagattattatcgcaacgccagcacag tggatttcccatacacaggatatttaaaaaaatatacattacagatcaacgtgtcaaa gtagttgcaggtggtacggatcgaaatgaaacaattatgaacattatcgaccatattcgc aatgtaaatggaattaataatgatgatgtgattgtaactcatgatgccgtaagaccattt ttaactcaacgtattattaaagagaacattgaagtagcagcaaaatatggtgcagtagat acagtcattgaagcaattgatacgattgtaatgtctaaagataaacagaacatacacagt atccctgtaaggaatgaaatgtatcaaggccaaacaccacaatcatttaatattaaatta ttacaagatagttatcgcgccttaagtagtgaacaaaaagaaatcttatcagatgcatgt aaaatcattgtcgaatctggacatgcagttaaattggtacgtggagaactatacaacatt aaagtgacaacaccgtatgatttaaaagtagcaaatgccattattcaaggtgatattgcc gatgat
168.	MEDNMIYAGILAGGIGSRMGNVPLPKQFLDIDNKPILIHTIRKFILVSEFNEIIIATPAQ WISHTQDILKKYNITDQRVKVVAGGTDRNETIMNIIDHIRNVNGINNDDVIVTHDAVRPF LTQRIIKENIEVAAKYGAVDTVIEAIDTIVMSKDKQNIHSIPVRNEMYQGQTPQSFNIKL LQDSYRALSSEQKEILSDACKIIVESGHAVKLVRGELYNIKVTTPYDLKVANAIIQGDIA DD

169.
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170.
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171.
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172.
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173.
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174.
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175.	atgcttttagtattagctggttgctctaattctaacgataataatgaaagtaaaaaagat gacgcagacaatggtaagaaacaagagattcaagttgcagcggcagcaagtttaacagat gtaaccaagaaattagcttcagaatttaaaaaagagcataaaaatgctgatattaaattt aactatggtggatcaggggcattaagaaaacaaattgaatcaggcgcacctgttgacgta tttatgtctgcaaatactaaagatgtagatgcattaaaagacaagaataaagcgcatgat acatataaatatgcgaaaaatagtctagtattaattggtgataaagattcaaattacact tcagtaaaagacttaaaagacaatgataaattagcattaggtgaagtgaaaactgtacca gcaggaaaatatgcgaaacagtatttagataacaataacttatttaaagaagtcgaaagt aaaatcgtttatgctaaagatgtaaaacaagtattaaattatgttgaaaagggtaatgcg aaacaaggttttgtgtataaaactgacttatataaacaaaataaaaaaattgatactgta aaagtaattaaagaagtagaacttaagaagccaatcacatacgaagctggtgctacatca gatagtaaattagcaaaagagtggatggaattcttaaaatcagataaagctaaagaaata ctaaaagaataccactttgcagca
176.	MLLVLAGCSNSNDNNESKKDDADNGKKQEIQVAAAASLTDVTKKLASEFKKEHKNADIKF NYGGSGALRKQIESGAPVDVFMSANTKDVDALKDKNKAHDTYKYAKNSLVLIGDKDSNYT SVKDLKDNDKLALGEVKTVPAGKYAKQYLDNNNLFKEVESKIVYAKDVKQVLNYVEKGNA KQGFVYKTDLYKQNKKIDTVKVIKEVELKKPITYEAGATSDSKLAKEWMEFLKSDKAKEI LKEYHFAA
177.	ttggcatacacatacactttaaaagatattattgaaattacaggtgtaactaaaagaact ttacattattacgatgaaataggattattagttccagataaaaatgataaaaattatcgc gtttataaacagcaagacttagaaaaattacaaaagattttaatactcaagtcttttgat tttgatatcgctaaaataaaacaatacatttcgtatgataatgaacaattgcgaaagtta ttatcagagcaaataagcaagttagataaaaagatttctgacttacaattaattagacgc tctgtatgtgaatttattaaaggactctctctaatagataccagcattttaaacaagaca ctacagtcacaatatgataaagaagcatctataaaatatggtcatacgaaagcatatcaa tcatttattagacgtaaagacagcttacaatcgcaggatatcagacataaattgacaact atcttcaataaatttaatcatatgtctttgagtcattatccaatccaagattgtagtgat ctcgtatttgagtggaaggcatttatgaacactatcgctgattttgatgatgaaacatta tgctgtattgctaaaacatatgaagatgatacgcgtttcaaagattactttaattcatat gataatcaaaatttagcatcatacatttcagaagctgttaattatttttgagcaatgtg aataagagcgacaatttt
178.	MAYTYTLKDIIEITGVTKRTLHYYDEIGLLVPDKNDKNYRVYKQQDLEKLQKILILLKSFD FDIAKIKQYISYDNEQLRKLLSEQISKLDKKISDLQLIRRSVCEFIKGLSLIDTSILNKT LQSQYDKEASIKYGHTKAYQSFIRRKDSLQSQDIRHKLTTIFNKFNHMSLSHYPIQDCSD LVFEWKAFMNTIADFDDETLCCIARTYEDDTRFKDYFNSYDNQNLASYISEAVNYFLSNV NKSDNF
179.	atggcaaaaataaaagcaaatgaagcattagttaaagcattacaagcatgggatatagat cacttgtatggtattccaggagactcaatcgacgcagtagtcgatagtttacgtacagtg agagatcaatttaaatttatcatgtacgtcatgaagaagtagcaagcttagcggctgct ggttacacaaaattaactggtaaaatcggtgtggcattaagtatcggtggccctggttta attcatttattaaatggtatgtatgatgccaaaatggataatgtaccgcaattaatatta tctggacaaacgaatagtacagcacttggaacgaaagcattccaagaaacaaatttacaa aaattatgtgaagatgtagccgtttataatcaccaaattgaaaaaggtgacaatgtgttt gaaatcgttaacgaagcaattcgtacggcatatgaacaaaaaggtgtagctgttgttatt tgtcctaacgacttattaactgaaaaaattaaagatacaacgaataaaccagtagataca tcaagaccaacagtagtatcaccaaaatataaagacatcaaaaaagcggttaaactaatt aataaaagtaaaaagcctgtcatgttaattggtgtaggtgcgaaacatgcgaaagatgag ctacgtgaatttattgaaatggctaaaattcctgtcattcattcattaccagctaaaaca atcttgccggatgatcatccatatagtatcggtaacttaggtaaaatcggtaccaaaaca tcttatcaaacaatgcaggaagcggatttattaattatggttggtacaaactatccatat gtggattacttacctaagaaaaatattaaagccattcaaattgacacaaatcctaaaaat atcggacatcgtttcaatattaatgtaggaattgttggagatagtaaaattgcgttgcat cagttaactgaaaatattaaacatgttgctgaaagaccattcttaaacaaaacgttagaa cgtaaagcggtttgggataaatggatggaacaagataaaaatataatagtaaaccatta cgtccagaacgattaatggcatcaatcaataaatttattaaagatgatgcagtgatttca gcagatgtaggtacagcaacagtttggtcaactcgatacttaaaccttggtgtaaataac aagttcatcatttcaagttggttaggtacaatgggttgcggtcttccaggtgcaattgca tcaaaaattgcatatccaaatagacaagccatcgcaattgctggtgacggtgcattccaa atggtaatgcaagacttcgctacagcagtacaatatgatttacctttaactgtatttgta cttaataacaaacagttagcatttattaaatatgaacaacaagcagctggtgaattagaa tatgcagttgatttttctgatatggatcatgcaaaatttgctgaggcagcaggtggtaaa ggttatacaattaagagtgctagcgaagtagatgctatagtcgaagaggcattagcacaa gatgtaccaacgattgtagatgtatatgttgatcctaatgctgcgccattaccaggtaaa attgtaaatgaagaagcgcttggttatggtaagtgggcatttagatcaattactgaagat aaacatttagatttagatcaaattccaccaatttcagtggcagcaaaacgtttctta
180.	MAKIKANEALVKALQAWDIDHLYGIPGDSIDAVVDSLRTVRDQFKFYHVRHEEVASLAAA GYTKLTGKIGVALSIGGPGLIHLLNGMYDAKMDNVPQLILSGQTNSTALGTKAFQETNLQ KLCEDVAVYNHQIEKGDNVFEIVNEAIRTAYEQKGVAVVICPNDLLTEKIKDTTNKPVDT SRPTVVSPKYKDIKKAVKLINKSKKPVMLIGVGAKHAKDELREFIEMAKIPVIHSLPAKT ILPDDHPYSIGNLGKIGTKTSYQTMQEADLLIMVGTNYPYVDYLPKKNIKAIQIDTNPKN IGHRFNINVGIVGDSKIALHQLTENIKHVAERPFLNKTLERKAVWDKWMEQDKNNNSKPL RPERLMASINKFIKDDAVISADVGTATVWSTRYLNLGVNNKFIISSWLGTMGCGLPGAIA SKIAYPNRQAIAIAGDGAFQMVMQDFATAVQYDLPLTVFVLNNKQLAFIKYEQQAAGELE YAVDFSDMDHAKFAEAAGGKGYTIKSASEVDAIVEEALAQDVPTIVDVYVDPNAAPLPGK IVNEEALGYGKWAFRSITEDKHLDLDQIPPISVAAKRFL
181.	gtagtagtttagggcttgcaacgcac
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212.	gtgaaagcattgaaattatatggcgtggaagatttacggtatgaggataatgaaaagcca gtcattgaaagtgcgaatgacgttattattaaagtacgagcgactggcatatgtggttca gacacgtcacgatacaaaaaaatggggccatacattaaaggtatgccatttggtcatgaa ttttcaggtgtagtagatgccattggaagtgatgttacgcatgttaatgtgggcgacaaa gtgacaggttgcccagcaatacctttgttatcaatgcgagtattgtttaaaaggtgaatat gcacgatgtgaaaagttattcgtcattggctcatatgaacctggatcgttcgcggaatat gtcaaattgccagcgcaaaatgttttaaaggttccagacaatgttgattacattgaagca gcaatggttgagccatcagccgttgttgcgcatgggttttataaatcgaatatacaacct ggtatgactgttgcagtaatggggtgtggcagtataggtttgttagctattcaatgggca cgaatatttggtgctgcacatatcatcgctatagatatagatgcgcataaactagatatt gcaacatcattgggcgcacatcaaacaatcaattcaaaagaagaaaatcttgagaaattc atcgaaaatcattacgccaatcaaatcgatttagctatagaatcatcaggtgctaaagtt acgattggtcaaatattgacgctacctaaaaaaggtggcgaggtggtattactcggaata ccatatgatgatattgagattgatcgcgttcatttgaaaaaattctgcgtaacgagttg acagtatgtggctcttggaactgtttgtccagtaattttccgggcaaagagtggacggca accttacattatatgaagacgaaagatattaatgtaaagcctattatttctcatttttta ccgttagaaaaaggcccggagacatttgataaattagttaacaagaaagaacgatttgat aaagtcatgtttacgatttat
213.	atgcaagcattacaaacatttaatttaaagagctaccagtaagaacagtagaaattgaa aacgaaccttatttgtaggaaaagatattgctgagattttaggatatgcaagatcagac aatgccattagaaatcatgttgatagcgaggacaagctgacgcaccaatttagtgcatca ggtcaaaacagaaatatgatcattatcaacgaatcaggattatacagtctaatcttcgat gcttctaaacaaagcaaaaacgaaaaaatcagagaaaccgctcggaaattcaaacgatgg gtaacatcagatgtcctaccagctattcgcaaacacggtatatacgcaacagacaatgta attgaacaaacattaaaagatccagactacatcattacagtgttgactgagtataagaaa gaaaaagagcaaaacttacttttacaacaagaaatcggagaactaaaacccaaagcagac tatgtagatgaaatcttaaagtcaactggcacattagccacaactcaaatcgcggcagac tacggtatatcagcacaaaagttaaacaaactactacacgaagctagactacaacgaaaa gtaaataaacagtgggtgctttactcagaacacatgggcaagagttacacagaatcagac actatagcaattgtacgctctgacggtagagaagacacagttttacaaactagatggaca caaaaaggcagattgaaaatacatgaaatcatgactgaattcggttatgaagctaattta gggggagcg
214.	atgaaattaaaatcattagcagtgttatcaatgtcagcggtggtgcttactgcatgtggc aatgatactccaaaagatgaaacaaaatcaacagagtcaaatactaatcaagacactaat acaacaaaagatgttattgctttaaaagatgttaaaacaagcccagaagatgctgtgaaa aaagctgaagaaacttacaaaggccaaaagttgaaaggaatttcatttgaaaattctaat ggtgaatgggcttataaagtgacgcaacaaaaatctggtgaagagtcagaagtacttgtt gctgataaaaataaaaaagtgattaacaaaaagactgaaaaagaagatacaatgaatgaa aatgataacttaaatatagcgatgctatagattacaaaaaagccattaaagaaggacaa aaagaatttgatggtgatattaaagaatggtcacttgaaaaagatgatggcaaacttgtt tacaatatcgatttgaaaaaaggtaataaaaaacaagaagttactgttgatgctaagaac ggtaaagtattaaagagtgagcaagatcac

215.	atgaaaatgaaaaatattgcaaaaataagtttgttattaggaatattagcaacaggtgta aacactacaacggaaaaaccagttcatgccgaaaagaaacctattgtaataagtgaaaat agcaaaaaattaaaagcttattataatcaacctagtattgaatataaaaatgtgacaggt tatatcagtttcattcaaccaagtattaaatttatgaatatcatagatggtaattctgtt aataatattgctttaattggcaaagataagcaacattatcatacgggtgtacatcgtaat cttaatatattttacgttaatgaggataagagatttgaaggtgcaaagtactctattggg ggtatcacgagtgcaaacgataaagctgtcgacctaatagcagaagcaagagttattaaa gaagatcatactggtgaatatgattatgacttttcccatttaaaatagataaagaagcg atgtcattgaaagagattgattttaaattaagaaaatacccttattgataattatggtctt tacggtgaaatgagtacaggaaaaattacagtcaaaaagaaatactatggaaagtataca tttgaattggataaaaagttacaagaagaccgtatgtccgatgttatcaatgtcacagat attgatagaattgaaatcaaagttataaaagca
216.	mrktkivctigpaseseemieklinagmnvarlnfshgsheehkgridtirkvakrldki vailldtkgpeirthnmkdgiielergnevivsmnevegtpekfsvtyenlindvqvgsy illddglielqvkdidhakkevkcdilnsgelknkkgvnlpgvrvslpgitekdaedirf gikenvdfiaasfvrrpsdvleireileeqkanisvfpkienqegidniaeilevsdglm vargdmgveippekvpmvqkdlirqcnklgkpvitatqmldsmgrnpratraeasdvana iydgtdavmlsgetaaglypeeavktmrniavsaeaaqdykkllsdrtklvetslvnaig isvahtalnlnvkaivaatesgstartiskyrphsdiiavtpseetarqcsivwgvqpvv kkgrkstdallnnavatavetgrvsngdliiitagvptgetgttnmmkihlvgdeiangq gigrgsvvgttlvaetvkdlegkdlsdkvivtnsidetfvpyvekalgliteengitsps aivglekgiptvvgvekavknisnnmlvtidaaqgkifegyanvl
217.	mqfdnidsalmalkngepiivvddenrenegdlvavtewmmdntinfmakearglicapv skdiaqrldlvqmvddnsdifgtqftvsidhvdtttgisayertltakklidpsseakdf nrpghlfplvaqdkgvlarnghteaavdlakltgakpagviceimnddgtmakgqdlqkf kekhqlkmitiddlieyrkklepeiefkakvkmptdfgtfdmygfkatytdeeivvltkg airqhenvrlhsacltgdifhsqrcdcgaqlessmkyinehggmiiylpqegrgiglink lrayelieqgydtvtanlalgfdedlrdyhiaaqilkyfniehinllsnnpskfeglkqy gidiaerievivpetvhnhdymetkkikmghli
218.	mkmkklvkssvassialllsntvdaaqhitpvsekkvddkitlykttatsdndklnisq iltfnfikdksydkdtlvlkaagninsgykkpnpkdynysqfywggkynvsvssesndav nvvdyapknqneefqvqqtlgysyggdinisnglsgglngsksfsetinykqesyrttid rktnhksigwgveahkimnngwgpygrdsydptygnelflggrqsssnagqnflpthqmp llargnfnpefisvlshkqndtkkskikvtyqremdrytnqwnrlhwvgnnyknqntvtf tstyevdwqnhtvkligtdsketnpgv
219.	mkkkallplflgimvflagcdyskpekrsgffyntfvdpmknvldwlgnnllndnyglai iilvlvirillpfmlsnyknshmmrqkmkvakpevekiqekvkrartqeekmaangelm qvykkydmnpiksmlgclpmliqlpiimglyfvlkdqlvdglfkyphflwfdlgrpdiwi tiiagvlyfiqayvssktmpdeqrqmgymmmvispimiiwislssasalglywsvsaafl vvqthfaniyyekvakkevqpfieayerehnggsnkkgkntqvvskkkk
220.	mnlfrqqkfsirkfnvgifsaliatvtfistnpttasaaeqnqpaqnqpaqpadantqpn anagaqanptaqpaapanqgqpavqpanqggqanpaggaaqpntqpagqgnqadpnnaaq aqpgnqatpanqagqgnnqatpnnnatpanqtqpanapaaaqpaapvaanaqtqdpnasn tgegsinttltfddpaistdenrqdptvtvtdkvngyslinngkigfvnselrrsdmfdk nnpgnyqakgnvaalgrvnandstdhgnfngisktvnvkpdseliinfttmqtnskqgat nlvikdakknteiatvnvaktgtahlfkvptdadrldlqfipdntavadasrittnkdgy kyysfidnvglfsgshlyvknrdlapkatnnkeytinteignngnfgaslkadqfkyevt lpqgvtyvnnsltttfpngnedstvlkmmtvnydqnankvtftsqgvttargthtkevlf pdkslklsykvnvanidtpknidfnekltyrtasdvvinnaqpevtltadpfsvavemnk dalqqqvnsqvdnshyttasiaeynklkqqadtilnedanhvktanrasqadidglvtkl qaalidnqaaiaeldtkaqekvtaaqqskkvtqdevaalvtkinndknnaiaeinkqtta qgvttekdngiavleqdvitptvkpqakqdiiqavttrkqqikksnaslqdekdvandki gkietkaikdidaattnaqveaiktkaindinqttpattakaaaleefdevvqaqidqap lnpdttneevaeaierinaakvsgvkaieatttaqdlervkneelskienitdstqtkmd aynevkqaatarkaqnatvsnatneevaeadaavdaaqkqglhdiqvvkskqevadtksk vldkinaiqtqakvkpaadtevenayntrkqeiqnsnastteekqaayteldtkkqeart nldaantnsdvttakdnsiaainqvqaattkksdakaeiaqkaserktaieamndsttee qqaakdkvdqavvtanadidnaaanndvdnakttneatiaaitpdanvkpaakqaiadkv qaqetaidgnngstteekaaakqqvqtekttadaaidaahtnaeveaakkaaiakieaiq patttkdnakeaiatkanerktaiaqtqditaeeiaaanadvdnavtqansnieaansqn dvdqakttgensidqvtptvnkkatarneitailnnklqeiqatpdatdeekqaadaean tengkanqaisaattnaqvdeakanaeaainavtpkvvkkqaakdeidqlqatqtnvinn dqnatteekeaaiqqlatavtdaknnitaatddngvdqakdagknsiqstqpatavksna kndvdqavttqnqaidnttgatteeknaakdlvlkakekayqdllnaqttndvtqikdqa vadiqgitadttikdvakdelatkaneqkaliaqtadatteekeqanqqvdaqltqgnqn ienaqsiddvntakdnaiqaidpiqastdvktnaraelltemqnkiteilnnnettneek gndigpvraayeeglnninaatttgdvttakdtavqkvqqlhanpvkkpagkkeldqaaa dkktqieqtpnasqqeindakqevdtelnqaktnvdqsstneyvdnavkegkakinavkt fseykkdalakiedaynakvneadnsnastsseiaeakqklaelkqtadqnvnqatskdd ievqihndldnindytiptgkkesattdlyayadqkknnisadtnatqdekqqaikqvdq nvqtalesinngvdngdvddaltqgkaaidaiqvdatvkpkanqaievkaedtkesidqs dqltaeektealamikqitdqakqqitdatttaevekakaqgleafdniqidstekqkai eeletaldqieagvnvnadatteekeaftnaledilskatedisdqttnaeiatvknsal eqlkaqrinpevkknaleairevvnkqieiiknadadasakeiartdlgryfdrfadkld ktqtnaevaelqnvtipaieaivpqndpdandtnngidnndatansnanatpentgqpnv settangkadaspttpnnsdaatgettatsatddandkpqannnssvdastnsptmdndv tskpevestnngttdkpvtetdnatpaesttnnnstttatnenaptgstatapttastea assadskdnasvndskqnaevnnsaesqstndkvaqpksenkakaekdgsdstnqsmves ttetlpsaditepnvpsntskdkeesttnqtdagqlksetnvasneadkspskadtevsn kpstsasseakekmtstnlsqkddtatadtndtqksvgsaannkatqndganaspatvsn gsnsanqdmlnvtntddhqaktksaqqgkvnkakqqaktlpdtgmshnddlpyaelalga gmaflirrftkkdqgtee
221.	mklksfvtatlalgllstvgaalpsheasadsnngykemtvdgybtvpytisvdgitalh rtyfifpenknvlyqeidskvknelasqrgvttekinnaqtatytltlndgnkkvvnlkk nddaknsidpstikqiqivvk
222.	maikkykpitngrrnmtsldfaeitkttpeksllkplpkkagrnnqgkltvrhhggghkr qyrvidfkrnkdginakvdsiqydpnrsanialvvyadgekryilapkglevgqivesga eadikvgnalplqnipvgtvvhnielkpgkggqiarsagasaqvlgkegkyvlirlrsge vrmilstcratigqvgnlqhelvnvgkagrsrwkgirptvrgsvmnpndhphgggegrap igrpspmspwgkptlgkktrrgkkssdklivrgrkkk

223.	mlvntfnpfdnlllssliaaipivlfllcltvfkmkgiyaaittlvvtlliaipffklpv giasgavvegffqgiipigyivmmavllykitvesgqfltiqdsitnisqdqriqvllig fafnaflegaagfgvpiaicallltqlgfnplkaamlclvanaasgafgaigipvgvvet lklpgdvsvlgvsqsatltlaiinfiipfllifiidgfrgvketlpailvvsitytltqg lltvfsgpeladiippllтmlalavfskkfqpkhiyrvnkdeeiepakahsakavlhaws pfivltvivmiwsapffknlflpngalsslvfkfnlpgtisevthkplvltlniigqtgt aillтiiitilmskkvnfkdagrlfgvtfkelwlpvlticfilaiskittygglsaamgq giakagnvfpvlspilgwigvfmtgsvvnnnslfapiqasvaqqigtsgsllvsantvgg vaaklispqsiaiataavkqvgkesellkmtlkysvcllificiwtfilsll
224.	mlknkilтttlsvsllaplanpllenakaandtedigkgsdieiikrtedktsnkwgvtq niqfdfvkdkkynkdalilkmqgfissrttyynykktnhvkamrwpfqyniglktndkyv slinylpknkiestnvsqilgyniggnfqsapslggngsfnysksisytqqnyvseveqq nsksvlwgvkansfatesgqksafdsdlfvgykphskdprdyfvpdselpplvqsgfnps fiatvshekgssdtsefeitygrnmdvthaikrsthygnsyldghrvhnafvnrnytvky evnwktheikvkgqn
225.	mkmnklvkssvatsmallllsgtanaegkitpvsvkkvddkvtlykttatadsdkfkisq iltfnfikdksydkdtlvlkatgninsgfvkpnpndydfsklywgakynvsissqsndsv nvvdyapknqneefqvqntlgytfggdisisnglsgglngntafsetinykqesyrttls rntnyknvgwgveahkimnngwgpygrdsfhptygnelflagrqssayagqnfiaqhqmp llsrsnfnpeflsvlshrqdgakkskitvtyqremdlyqirwngfywaganyknfktrtf kstyeidwenhkvklldtketennk
226.	mnremlylnrsdieqaggnhsqvyvdaltealtahahndfvqplkpylrqdpenghiadr iiampshiggehaisgikwigskhdnpskrnmerasgviilndpetnypiavmeasliss mrtaavsviaakhlakkgfkdltiigcgligdkqlqsmleqfdhiarvfvydqfseacar fvdrwqqqrpeinfiatenakeavsngevvitctvtdqpyieydwlqkgafisnisimdv hkevfikadkvvvddwsqcnrekktinqlvlegkfskealhaelgqlvtgdipgreddde iillnpmgmaiedissayfiyqqaqqqnigttlnly
227.	mkkimvifgtrpeaikmaplvkeidhngnfeanivitaqhrdmldsvlsifdiqadhdln imqdqqtlagltanalakldsiineeqpdmilvhgdttttfvgslaafyhqipvghveag lrthqkyspfpeelnrvmvsniaelnfaptviaaknllfenkdkerifitgntvidalst tvqndfvstiinkhkgkkvvlltahrrenigepmhqifkavrdladeykdvvfiypmhrn pkvraiaekylsgrnrieliepldaiefbnftnqsylvltdsggiqeeaptfgkpvlvlr nhterpegveagtsrvigtdydnivrnvkqlieddeayqrmsqannpygdgqasrricea ieyyfglrtdkpdefvplrhk
228.	mimgnlrfqqeyfriyknntestthrnaywvklaknveatkmmyalstivqqhasirhff dvttddnltmilheflpfieikqvpsssanydleaffkqelstyhfndsplfkvklfqfa daayilldfhvsifddsqidifldldcnayrgntvinntrqhahinrnddkdnqdashia ldsnyfrlennsdihidsyfpikhpfeqalyqtyliddmtsidmaslavsvylanhimsq qhdvtlgihvpshlpndlhquivpltltidakdvcqrfttdfnkcvlqnmsqlqcakssl sletifhcyhbmmsccndviedvhqihdahtsladieifphqhgfkiiynsaaydllsie tlsdlvrniylqiteengnkrttvdelnlmterdiqlyddinlslpeiddaqtvvtlfeq qveatpnhvavqfdgvfityqtlnarandlahrlrnqygvepndrvaviaeksiemiiam igvlkaggayvpidpnypsdrqeyilkdvtpkvvityqalyengkqninhidlnkiawkn idnlskcntledhayviytsgttgnpkgtliphrgivrlvhqnhyvplneettillsgti afdaatfeiygallnggklivakkeqllnpiaveqlinendvntmwltsslfnqiaseri evlvslkylliggevlnakwvdllnqkpkhpqiingygptenttfttynipnkvpnrip igkpilgthvyimqgerrcgvgipgelctsgfglaagylnqpeltadkfikdsninqlmy rsgdivrllpdgnidylyrkdkqvkirgfrielsevehaleriqginkavvivqnhdqdq yivayyeamhtlshnkiksqlrntlpeymipvnfmhieqipitingkldkkalpimdyvd tdayvapstdtehllcqifadilhvnqvgihdnffelgghslkatlvvnrieastgkrlq igdllqkptvfelaqaiakvqeqnyevipetivkddyvlssaqkrmyllwksnhkdtvyn vpflwrlsselnvaqlrqavqrliarheilrtqyivvddevrqrivadvavdfeevnthf tdeqeimrqfvapfnlekpsqirvryirsplhaylfidthhiindgmsniqlmndlnaly qhkllplklqykdysewmshrdmtkhrqywlsqfkdevpilslptdyvrpnikttngam msftmnqqmrqllqkyvekhqitdfmffmsvvmtllsryarkddvvvgsvmsarmhkgte qmlgmfantlvyrgqpspdkmwtqflqevkemsleayehqeypfeclvndldqshdasrn plfdvmlvlqnnetnhahfghsklthiqpksvtakfdlsfiieedrddytinieyntdly hsetvrhmgnqcmimidyilkhqdtlqicdipngteellnwvnthvndrmlnvpgnksii syfnevvsrqgnhvalvmndltmtyetlrnyvdaiahmllsngvgngqrvalftersfem iaamlatvkvgasyipididfpnkrqgailedakvtavmsygveiettlpviqlenakgf veskeneqyddlhgnqlentamldnemyaiytsgttgmpkgvairqrnllnlvhawstel qlgdnevflqhanivfdasvmeiycclingntlvipdreervnpeqlqqlinkhrvtvas iplqmcsvmedfyieklitggatstasfvkyiekhcgtyfnaygpsestvitsywshhcg dlipetipigkplsniqvyimsdglicgigmpgelciagdslaigyinrpelmadkwqnn pfgkgklyhsgdlarytsdgqieflgridkqvkvngyrieldeienailairgisdcvvt vshfdthdilnayyvgeqqveqdlkqylndqlpkymipktithidcmpttndkvdttrl pnpspiqqsnkvysepsneieqtfvdvfgevlkqndvgvdddffelggnsleamlvvshl krfghhismqtlyqyktvrqivnymyqnqqslvalpdnlselqkivmsrynlgiledsls hrplgntlltgatgflgaylievlqgyshriycfiradneeiawyklmtnlndyfseetv eimlsnievivgdfecmddvvlpennmdtiihagartdhfgdddefekvnvqgtvdvirla qqhharliyvstisvgtyfdidteavtfseadvykgqlltspytrskfyselkvleavnn gldgrivrvgnltnpyngrwhmrniktnrfsmvmndllqldcigvsmaempvdfsfvdtt arqivalaqvntpqiiyhvlspnkmpvkslecvkrkeielvsdesfneilqkqdmyeti gltsvdreqglamidttltlkimnhisekwptitnnwlyhwaqyiktifnk
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230.	mskilkcitlavvmllivtacgpnrskedidkalnkdnskdkpnqltmwvdgdkqmafyk kitdqytkktgikvklvnigqndqlenisldapagkgpdifflahdntgsaylqglaaei klskdelkgfnkqalkamnydnkqlalpaivettalfynkklvknapqtleeveanaakl tdskkkqygmlfdaknfyfnypflfgnddyifkkngseydihqlglnskhvvknaerlqk wydkgylpkaathdvmiglfkegkvgqfvtgpwnineyqetfgkdlgvttlptdggkpmk pflgvrgwylseyskhkywakdlmlyitskdtlqkytdemseitgrvdvkssnpnlkvfe kqarhaepmpnipemrqvwepmgnasifisngknpkqaldeatnditqnikilhpsqndk kgd
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245.

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267.

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268.	mtkkekdykksleqqktrvkiyksgkswvkasineiellktmglpflskneiqenvtekt kghklkksaakttalvggaftfnmlnnhqafaasetpitseissnsetvanqnsttikns qketvnstslesnhsnstnkqmssevtntaqssekagisqqssetsnqsskintyastdh vesttinndntaqqdqnkssnvtskstqsntssseknissnltqsietkatdslatsear tstnqisnltststsnqssptsfanlrtfsrftvlntmaapttttsttttssltsnsvvvn kdnfnehmnlsgsatydpktgiatltpdaysqkgaislntrldsnrsfrfigkvnlgnry egyspdgvaggdgigfafspgplgqigkegaavgigglnnafgfkldtyhntstprsdak akadprnvggggafgafvstdrngmatteestaaklnvqptdnsfqdfvidyngdtkvmt vtyagqtftrnltdwiknsggttfslsmtastggaknlqqvqfgtfeytesavakvryvd antgkdiippktiagevdgtvnidkqlnnfknlgysyvgtdalkapnytetsgtptlklt nssqtviykfkdvqgpqisvdsqtrevgktinpititttdnskdvltttvtglpsglsfd qttntiigtpsevgtttvtvnttdatgnvtskqftitiqdtispvvnvtpsqasevftpi npititatdnsgkvvthtvtglpqglkfdastnsivgtptqigtntitiestdasgnktt tkinyevtrnsasdststsivnsvstsisnstslsdsvkasqslstkestskslsgslsa stsnsasikasesastskklsesastsmsdsasikasesastskklsesaststsdsasi kasesastskklsesastsmsdsvsikasesastskklsesaststsdsastqasesast skklsestststsdsaststsesdstsestslsdstsaslsestststsdsaststsesd snststslsestststslsestststsdsastsasvsdsnsastslrestststsdsststst sdsaststsesdsdsastslsgssstsvsdstsastsesastststisdsnststslsest stslsestststsdsastsmsvsdsnsasislseststsvsdststststsesastststesd snsastslsestststvsdsstststsdsaststsvsdsnsastslsestststisdsststst sdsaststsvsessstskklsesastsmsdsasastsesnststslsgststslsgstst stsesaststsvsdsnsastslsestststslsdststststsdstsastsesdsnststsmse slstsvsdstststsdsaststsvsdsnsastslsdststsisdstsastsdsasesase sestsestsvsessstsvsdststststsesastststsesestsestsvsessstsvsdstst stsesaststsesestsestslsgssstsvsdststststsesaststsvsdsnststslse ststslsnstststsdsaststsvsdsnsastslsgslstnvsdststststsdsaststse sdsnsastslsgslstsisdststststsdsaststsesaststsvsesdsestsmsvsdsn ststslsdststsvsdstrtstsdsastsksvsesnsastsvsgststsisdsststsm stsetftsqspinsesqfigdslsedtivtqsknt nmlnktgkdydlqeqrgytdseqhn etqsnqadnhsnnldllhqnrlqdkvvkqptkgedgvvsngfivavaivlaifglakksr kddddqdgsk
269.	mkktviastlavslgiagyglsgheahasettnvdkahlvdlaqhnpeelnakpvqagay dihfvdngyqynftsngsewswsyavagsdadytesssnqevsantqssntnvqavsapt ssesrsyststtsysapshnysshsssvrlsngntagsvgsyaaaqmaartgvsastweh iiaresngqlharnasgaaglfqtmpgwgstgsvndqinaaykaykaqglsawgm
270.	mnknkvivigstnvdkflnvkrfpkpgetlhinqaqkefgggkganqaiaasrlaadttf iskvgkdgnanfiledfkkagihtqyiltseseetgqafitvdeagqntilvyggannmtl satdvemsvdafigadfvvaqlevpfeaieqafkiarkqnittvlnpapaielpksllel tdiiipneteaelltgisinnesdmketatyfldlgisavlitlgeqgtycayqeqykmi pacnvkaidttaagdtfigaflselnkdlsnlesairlanqassltvqrkgaqasiptrk eveaeyn
271.	malkkykpitngrrnmttldfaeitkttpeksllqplpkragrnnqgkltvrhhggghkr qyrvidfkrnkdgiiakvdsiqydpnrsaniallvyadgekryiiapkglqvgqtvesga eadikvgnalplqnipvgtvihnielkpgkggqlarsagassqvlgkegkyvlirlrsge vrmilstcratigqvgnlqhelvnvgkagrsrwkgvrptvrgsvmnpndhphgggegrap igrpspmspwgkptlgkktrrgkkssdklivrgrkkk
272.	mkskftillftifsttvlvlviiynktqsqsyisthysnnkikttatlflhgyggserse tfmvkqalnknvtnevitarvssegkvyfdkklsedaanpivkvefkdnkngnfkenayw ikevlsqlksqfgiqqfnfvghsmgnmsfafymknygddrhlpqlkkevniagvyngiln mnenvneiivdkqgkpsrmnaayrqllslhkiycgkeievlniygdledgshsdgrvsns ssqslqylirgstksyqemkfkgakaqhsqlhenkdvaneiiqflwet
273.	mkigidaggtlikivqehdnrryyrtelttniqkvidwlnneeietlkltggnagviadq ihhspeifvefdasskgleilldeqghqiehyifanvgtgtsfhyfdgkdqqrvggvgtg ggmiqglgyllsnitdykeltnlaqngdrdaidlkvkhiykdteppipgdltaanfgnvl hhldnqftsanklasaigvvgevittmaitlareyktkhvvyigssfnnnqllrevveny tvlrgfkpyyiengafsgalgalyl

274.	mtlnnhfaytfeerptpklwlckpdgtrieriadfsklggtfkftnvntlhfdlplqvfs edtkqiernkvvdlvknkylidyryngyrdifviddikksandsdfitlnldsraselnk kaaneiellgstipqmmnkilsvyaplwklghvdgkiidvkreltgsnttvnalidnics lfdavaiynninrtisfytkdnvgtnrglrvrensylksfedqfvskdivtrlypfgqsg ltiqsvnpagssyiedfsyfmspfkrdnnrnvlqhsdymsdelchalldyqefyaskkdq agelskqysailkehsqedfrlnqlsatlqrlnervelvkpkseyidlgtkvknfkitvp kssyylimirndgsftrikfnnkqydipsgewlyiklktgkfndatkfekqleypleils ananlrvvytrssegdyeeedtktieekynlekykilvkdqekvvasierrlkafedqka svirsmnaknflseklynerelyvfesvwteenhtdaqelyddavkqmkeqkkinrtitv dlvnfiqsldhkddwdklnvgdkvvfqnkifntkikayitemqldfqtnqvkitsdifd ykdldtiiaeklaqttstssqvdfhkqqireqtgritdmtrliegewdankkrvmagnet vdigshgvkviskenpnefvimvggviamtrdngetfktgitpeginaemligkmivget ltfenesgtvkfdkdglyvnsknfhlvsndgeedyfdklkremsenakqqtdrmleeykk evsqtiseatdvrnivdnaadilqaafadgvitdvekrlisetlaqlekenrefedkinl alnhpyiteedtielnnsiveyssmyetlvisinesvsdkmitpqeseeinqniinfree ikdilslveeiiertknaqlqatleeakdyttrvrddikdelkdlnnsfkslnstveesl qdnifdaaeleaiktvvlvtkseyqditnryssmsantdlkseskldltksyktldtsfn dfvkyidemtmdriadetekvnykkkydtlqknlsdymkkydncileiskkysndaadkv lgdftaiatelqndfqdvkdnwaefkqttlesfkdgivteaekarlrvqldmldresmdi eeryksllanqytntdiknrltasrspylsvhaslrkvieqiiadgkvdesektlannsl ntynttltayskticqealntlsqiissdvaskkveefngvittissdvdtikkqrdgavi tyyysgvptlsndpakswttndlkdlhikdmyldtksgyaytftksgtsyswkpltdqvi vsslkqaknaqdtadnkrrvfvtqpippydqggdmwtqgsqgdiyvcgtsratgsfvssdw vkaskytddtvakqaakdledykvkmtkdfkdlndgvstfktevvkdfkdgivteaektr lrvqldildresqdieerynsifnsqyadtqvktsisnarstymnsltklrntiqtvied gkvtptekttanqtltaynnaltsysaaiqealnsmskviaqkeatsqvnqfneviknin tnitdiqkqvdgaietfyysgvptltnipasywttaskreahlgdlyldtatgvayrflk kgttsptyywspisdqiitdalnraktaqdtadgkrrvfvntpvppydtgdmwtqgasgd ilvcktpkakggiysisdwvkaskytddtvansavqqlneykrtnnldiadlkrktsdfe ktvvnafddrvisisesssikgqlallnhekdrltrqveniirnsnlvgaektklstays nintklsdlsttinsaivdnkivdaesksvtskfelykasvneyqiafdnalnsiireia ssqakdrldewkrtefstdsdgiiervagakfdskwtdtwrntvnpaiqqvsnitygsen lllnsesrsdgantttbsfiryyltrpletgktytlkasvlttderqsgqisvypyspng aretvnikdgkityttftaqtestqfliykdvagqsdvdlnvtiekailvegnkvtgwspa peetssalrdyntrissaetfiekmkekisqiatksdvdaslskvatyetqynvssgtny qiplqeyngsfftdnytyevvaknnslssnnvataifvskgsnngyelveldmmsktgan prfvldskgrpsistfspqsttqdisviytkylgsasainttkslieqtassielqvkkl taeteynnillnsdfssgwegwinvdpqysivdkntfgitlpdaitnkkkyntvkmtyn kntnypsvfsnfisvgkgqevaigehltltcyayipssskgkltgniyiefagyyekdqk snpmiarheilpkdfeynkwfrmtastaipstnsegkkinyiraclrydgknqsvnnsai fyyalpqlergskptewslsrldvfsteqlaakialnpesvdiiarnidfntdsmkiyns ngtlnisgdtltisnnnssneviinpkgftlkkdgvvkfkngldtsdysvqayepqfssw nnikatdpaakskynyirhiepgmngyytintgvynfavqhkqllevnntknarvnryty lynkrylkiqmsassrgkskiyiifkttkgdttlhqeivssssmvypditidlqaklgyp pnnlpdffelqagiaygennsidgffrirrmamtdtpnaev
275.	mynvtqhatyktknkretavligvhaqtdrqfnfestmeeldalsqtcqlnvkgqitqnr eqfdhkyyvgkgkideiksfiefhdidvvvtndelttaqsktlndnlgikiidrtqlile ifalrarsregklqvelaqldyllprlhghgkslsrlgggigtrgpgetklemdrrhirt rmneikhqlktvvdhreryrnkreqnqvfqialvgytnagksswfnvlaneetyekmilf atldpktrqiqvnegfnliisdtvgfiqklpttlvaafkstleeakgadvlmhvvdashs eyrtqidtvnqiindldmdhipqvvifnkkdlcneqmdvpvsksahvfvssrdendkqkv knlviqeiknslspyeeivdsadadrlyflkqhtlvtelifdetqasyrikgfkkl
276.	mmiivmlilsyligafpsgliigklffkkdirqygsgntgatnsfrvlgrpagfivtfld ifkgfitvffplwfpvhadgvistfftnglivglfailghvypiylkfnggkavatsagv vlgvnpilllllaiiffsvlkifkyvslssiiaaiscvigsiiihdyillavsgivsiil iirhksnivrifkgeepkikwm
277.	mmnhsealteqvfsfaselyaygvrevvispgsrstplalvfeahpniktwihpdersaa ffalglikgsekpvailctsgtaaanytpaiaesqisrlplvvltsdrphelrsvgapqa inqvnmfsnyvnfqfdlpiadgsehtidtinyqmqiasqylygphrgpihfnlpfreplt pdldrvdlltsvtktlphyqksisvddikdilqekngliivgdmqhqavdqiltystiyd lpiladplsqlrkekhpnvittydllyraglnlevdyvirvgkpviskklnqwlkktday qiivqnndqidvfptpphisyeisandffrslmeeplverkkwlqqwqsleqqarieisd ylkhatdeaayvgsliqkltkedtlfvgnsmpirdvdnllfdseasvyanrgandigdvv stalgmaahknvtlligdlsfyhdmngllmaklnelhinivlvnnngggifsylpqkrsa tkyferlfgtptglnfeytallydftfkrfdnltdfkyaelskmgshmyevitnrdenlh qhqnlyqklseivnvtl
278.	makkfnyklpsmvaltlfgtaftahqanaaeqpqnqsnhknvlddqtalkqaekaksevt qsttnvsgtqtyqdptqvqpkqdtqsttydasldemstyneissnqkqqslstddanqnq tnsvtknqqeetndltqedktstdtnqlqetqsvakenekdlgananneqqdkkmtasqp senqaietqtasndnesqqksqqvtseqnetatpkvsntnasgynfdyddeddssstdhl epislnnvnatskqttsykykepaqrvttntvkketasnqatidtkqftpfsataqprtv ysvssqktsslpkytpkvnssinnyirkknmkaprieedytsyfpkygyrngvgrpegiv vhdtandnstidgeiafmkrnytnafvhafvdgnriietaptdylswgagpygnqrfinv eivhthdydsfarsmnnyadyaatqlqyynlkpdsaendgrgtvwthaaisnflggtdha dphqylrshnysyaelydliyekyliktkqvapwgttstkpsqpskpsggtnnkltvsan rgvaqikptnnglyttvydskghktdqvqktlsvtktatlgnnkfylvedynsgkkygwv kqgdvvyntakapvkvnqtynvkagstlytvpwgtpkqvaskvsgtgnqtfkatkqqqid katylygtvngksgwiskyylttaskpsnptkpstnnqltvtnnsgvaqinaknsglytt vydtkgkttnqiqrtlsvtkaatlgdkkfylvgdyntgtnygwvkqdeviyntakspvki nqtynvkpgvklhtvpwgtynqvagtvsgkgdqtfkatkqqqidkatylygtvngksgwi skyyltapskvqalstqstpapkqvkpstqtvnqiaqvkannsgirasvydktaksgtky anrtflinkqrtqgnntyvllqdgtsntplgwvnindvttqnigkqtqsigkysvkptnn glysiawgtknqqllapntlanqafnaskavyvgkdlylygtvnnrtgwiaakdliqnst daqstpynytfvinnsksyfymdptkanryslkpyyeqtftvikqkningvkwyygqlld gkyvwikstdlvkekikyaytgmtlnnainiqsrlkykpqvqneplkwsnanysqiknam dtkrlandsslkyqflrldqpqylsaqalnkllkgkgvlenqgaafsqaarkygineiyl ishalvetgngtsqlakggdvskgkfttktghkyhnvfgigafdnnalvdgikyaknagw tsvskaiiggakfignsyvkagqntlykmrwnpanpgthqyatdinwanvnaqvlkqfyd kigevgkyfeiptyk

279.	vafefrlpdigegihegeivkwfikagdtieeddvlaevqndksvveipspvsgtveevl vdegtvavvgdvivkidapdaeemqfkghgddedskkeekeqespvqeeasstqsqekte vdesktvkampsvrkyarengvnikavngsgkngritkedidaylnggsseegsntsaas estssdvvnasatqalpegdfpettekipamrkaiakamvnskhtaphvtlmdeidvqel wdhrkkfkeiaaeqgtkltflpyvvkalvsalkkypalntsfneeagevvhkhywnigia adtdkgllvpvvkhadrksifeisdeinelavkardgkltseemkgatctisnigsaggq wftpvinhpevailgigriaqkpivkdgeivaapvlalslsfdhrqidgatgqnamnhik rllnnpelllmeg
280.	mnetdeisqiynkhrlpslsglakvsplvhrasiggvlnvaelnrikrlvqvqnqfktfy nqmleedeevkypilhdkmnhlpiltdlfkeinetcdahdlfdhasytlqsirskisrtn qrirqnldrivknqgnqkklsdaivtvrndrnvipvkaeyrqdfngivhdqsasgqtlyi epnsvvemnnqisrlrndeavererilteltgfvsaeadalliaesvmgqidfliakary artikgtkptfkedrtiylpnafhplldkdtvvantiefiddvetviitgpntggktvtl ktlgliivmaqsglliptldgsqlsifenvycdigdeqsieqslstfsshmkniveilqd adqnslilfdelgagtdpsegaalamsildyvrrlgslvmatthypelkaysynregvmn asvefdvetlsptykllmgvpgrsnafdiskklglslniinkaktmigtdeqeinamies leqnskrvdqqrieldrlvreaqqthdalskqyqqyqnyetslmdeakekanqrvksatk eadeilkelrnlrdhkgaevkehelidkkkqlddqyevksikqhvqkkkydtihtgdevk vlsyggkgevlelvgdeeavvqmgiikmklpiedlektkkkkekptkmvtrqnrqtikte ldlrgyryeealneldqyldqavlsnyeqvyiihgkgtgalqkgvqqhlkkhksvrqfrg gmqseggfgvtvaelk
281.	msffkrlkdkfssknedd iqkdldesvdsnvnsdsdsmdpndsdeqvkpkkkpkklsead fdedglisiedfeeieaqkigakfkagleksrqnfqeqlnnliaryrkvdedffealeem litadvgfntvmkltdelrteaqrrniqetedlrevivekiveiyhqeddhseamniedg rlnvilmvgvngvgkttigklayryqqegkkvmlaagdtfragaiqqlnvwgervgvev vsqnegsdpaavvydainaaknkdvdilicdtagrlqnksnlmqeldkmkrvinraipda pheallcldattgqnalsqarsfkevtnvsgivltkldgtakggivlairnelhipvkyv glgekmddlqpfspesyvyglfadmieqnedipeeisrnssveseegn
282.	mkrnwwkeavayqvyprsfndsngdgigdlpgliekldylenlgidviwlspmypspndd ngydisdykgimsefgtmndfdqllssihqrgmklildlvvnhtsdehpwfieskssktn akrdwyiwadpkpdgsepnnwesifngstwefdestkqyyfhlfskkqpdlnwenpdvrq avfemmnwwfekgidgfrvdaithikknfeagdlpvpdgkkfapafdvdmnqpgiqewlq emkdkslsrydimtvgeangvtpndaeewvgeengkfnmifqfehlglwstgdtkfdvks ykqvlnrwqkqlenvgwnalfienhdqprrvstwgddknywyesatshatayflqqgtpf iyqgqeigmtnypfesiesfndvavkteyqivkkeggdvnqlldkykmenrdnartpmqw nnsinagfttgkpwfhvnpnyteinvkqqlndkfsilsyykaliqlkksdliytygkfnm vdaenkqvfaytrtfknntvliivanltnevselnlpfeldissvdiklhnyhlndinldh ikpyesfvvei
283.	lshrklfpsifhlyqqdnldehiaiigigrrdynneqfrdqvkasiqtyvkdtdridefm thvfyhktdvsdkesyqsllqfserldsefalggnrlfylamapqffgvisdylkssglt qttgfkrlvlekpfgsdlksaeslnnqirrsfkeeeiyridhylgkdmvqnievlrfana mfeplwnnkyisniqvtssevlgvedrggyyessgalkdmvqnhmlqmvallameapisl nsedlraekvkvklkslrqlkpeevkknfvrgqydqgnidgkqvksyreedrvakdsvtpt fvsgkltidnfrwagvpfyirtgkrmksktiqvvvefkevpmnlyyetdnlldsnllvin iqpnegislhlnakkniqgidtepvqlsyamsaqdkmntvdayenllfdclkgdatnfth weelkstwkfvdaiqdqwtmvepcfpnyeagtngplesdlllsrdgnhwwddih
284.	mikknkeelndmeylvtqengteppfqneywnhfekgiyvdklsgkplftsedkfesncg wpsfskalnddeivelvdksfgmirtevrsekanshlghvfndgpkekgglrycinsaai qfipydkleelgygdlikhfkk
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286.	mariatklgypesnsfvtntviefvlhneayprlyriktrdtnlikisqaneisrqitng tmtleeakyqleeiyvakrdsslpfkgiaaaiiatsflylqggrlvdiitavlagtigyl vveildrklhaqfipefigslvigiisvighafvpsgdlatiiiaavmpivpgvlitnai qdlfgghmlmfttkslealvtafgigagvssililv
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327.	minavviavilmimlclcrlnvvislfisalvgglisgmsiekvinvfgknivdgaeval syallggfaalisysgitdylvgkiinaihaensrwsrvkvkvtiiiallamsimsqnli pvhiafipivipplslslfndlkidrrligliigfglcfpyvllpygfgqifqqiiqsgfa kanhpiefnmiwkamlipsmgyivglliglyvyrkpreyetrkisdsdnvtelkpyiliv tivailatflvqtftdsmifgalagvlvffisraynwyeldakfvegikimayigvvilt angfagvmnatgdidelvktltsitgdnklfsiimmyviglivtlgigssfatipiiasl fipfgasigldtmalialigtasalgdsgspasdstlgptaglnvdgqhdhirdtcvpnf lfyniplmifgtiaamvl
328.	mnhnviivialiivvismlamlirvvlgpsladrvvaldaiglqlmavialfsillniky mivvimmigilaflgtavfskfmdkgkviehdqnhtd
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331.	mentinesekkkrfklkmpgafmilfiltvvaviatwvipagayskl syepssqelkivn phnqvkkvpgtqqeldkmgvkikieqfksgainkpvsipntyerlkqhpagpeqitssmv egtieavdimvfilvlggligvvqasgsfesgllaltkktkghefmlivfvsilmiiggt lcgieeeavafypilvpifialgydsivsvgaiflassvgstfstinpfsvviasnaagt tftdglywrigacivgaifvisylywyckkikmdpkasysyedkdafeqqwsvlkdddsa hftlrkkiiltlfvlpfpimvwgvmtqgwwfpvmasafliftiiimfiagtgksglgekg tvdafvngasslvgvsliiglarginlvlnegmisdtilhfssslvqhmsgplfiivllf iffclgfivpssslglavlsmpifapladtvgiprfvivttyqfgqyamlflaptglvmat lqmlnmryshwfrfvwpvvafvlifgggvlitqvliys
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336.	mnllkknkysirkykvgifstligtvlllsnpngaqalttdnnvqsdtnqatpvnsqdkd vannrglansaqntpnqsattnqatnqalvnhnngsivnqatptsvqsstpsaqnnnhtd gnttatetvsnannndvvsnntalnvptktnengsgghltlkeiqedvrtssnkpelvai aepasnrpkkrsrraapadpnatpadpaaaavgnggepvaitapytpttdpnannagqna pnevlsfddngirpstnrsvptvnvvnnlpgftlinggkvgvfshamvrtsmfdsgdnkn yqaqgnvialgrihgtdtndhgdfngiekaltvnpnselifefntmttkngqgatnviik nadtndtiaektveggptlrlfkvpdnvrnlkiqfvpkndaitdargiyqlkdgykyysf vdsiglhsgshvfverrtmdptatnnkeftvttslknngnsgasldtndfvyqvqlpegv eyvnnsltkdfpsnnsgvdvndmnvtydaanrvitiksatgggtansparlmpdkildlry klrvnnvptprtvtfnetltyktytqdfinsaaeshtvstnpytidiimnkdalqaevdr riqqadytfasldifnglkrraqtildenrnnvplnkrvsqayidsltnqmqhtlirsvd aenavnkkvdqmedlvnqndeltdeekqaaiqvieehkneiignigdqttddgvtrikdq giqtlsgdtatpvvkpnakkairdkatkqreiinatpdvtedeiqdalnqlatdetdaid nvtnattnadvetaknngintigavvpqvthkkaardainqatatkrqqinsnreatqee knaalneltqatnhaleqinqattnadvdnakgdglnainpiapvtvvkqaardavshda qqhiaeinanpdatqeerqaaidkvnaavtaantnilnantnadveqvktnaiqgiqait patkvktdaknaidksaetqhntifnnndatleeqqaaqqlldqavatakqninaadtnq evaqakdqgtqnivviqpatqvktdtrnvvndkareaitninattgatreekqeainrvn tlknraltdigvtsttamvnsirddavnqigavqphvtkkqtatgvlndlatakkqeinq ntnatteekqvalnqvdqelatainninqadtnaevdqaqqlgtkainaiqpnivkkpaa laqinqhynaklaeinatpdatndeknaaintlnqdrqqaiesikqantnaevdqaatva ennidavqvdvvkkqaardkitaevakrieavkqtpnatdeekqaavnqinqlkdqainq inqnqtndqvdttqavnaidnveaevvikpkaiadiekavkekqqqidnsldstdnek evasqalakekekalaaidqaqtnsqvnqaatngvsaikiiqpetkvkpaarekinqkan elrakinqdkeataeerqvaldkinefvnqamtditnnrtnqqvddttsqaldsialvtp dhivraaardavkqqyeakkreieqaehatdeekqvalnqlannekralqnidqaiannd vkrvetngiatlkgvqphivikpeaqqaikasaenqvesikdtphatvdeldeanqlisd tlkqaqqeientnqdaavtdvrnqtikaieqikpkvrrkraaldsieennknqldairnt ldttqderdvaidtlnkivntiknditaqnktnaevdrtetdgndnikvilpkvqvkpaar qsvgvkaeaqnalidqsdlsteeerlaakhlveqalnqaidqinhadktaqvnqdsinaq niiskikpattvkatalqqiqniatnkinlikanneatdeeqniaiaqvekelikakqqi asavtnadvayllhdekneireiepvinrkasareqlttlfndkkqaieaniqatveern silaqlqniydtaiggidqdrsnaqvdktaslnlqtihdldvhpikkpdaektinddlar vtalvqnyrkvsnrnkadalkaitalklqmdeelktartnadvdavlkrfnvalsdieav itekensllridniaqqtyakfkaiatpeqlakvkvlidqyvadgnrmidedatlndikq htqfivdeilaiklpaeatkvspkeiqpapkvctpikkeethesrkvekelpntgsegmd lplkefalitgaallarrrtknekes
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342.	mmygypekwlegmttgegiaaelrlgivnghiaegtlltenqmakqfnvsrspirdafkl lqqnqliqlermgahvlpfgeqekkemydlrlmlesfafsrvknqerlpivkemkkqlem mkvavkfedaesftkhdfefhetlikasnhqylnsfwshlkpvmmalvltsmrqrmqqnp qdferihhnhqvfidaveqydsqilkeafhlnfddvgkdiegfwln
343.	mgsffnkiarkedpaiyqnkdghlkrtlrvrdflalgvgtivstsiftlpgivaaehagp avalsfllaaivaglvaftyaemaaampfagsayswvnvlfgeffgwvagwallaeyfia vafvasgfsanlrglvkpigielpaalsnpfgtnggfidiiaaivilltalllsrgmsea armenilvilkvlaiilfvivgltainvsnyvpfipehkvtatgdfggwqgiyagvsmif layigfdsiaansaealdpqktmprgilgslsvaivlfiavalvlvgmfhysqyannaep vgwalrqsghgvvaaivqaisvigmftaligmmlagsrllysfgrdgllpswlshlndkh lpnralviltiigvligsmfpfaflaqlisagtlvafmfvslamyrlrkregkdlpipaf klplypvlpaitfvlvllvfwglgfeaklytliwfivgiilylsyglrhskkndvaeyhp pk

344.	mnsdnmwltvmgliiiisivglliakkinpvvgmtiipclgamilgysvtdlvgffakgl dqvinvvimfifaiiffgimndsglfkplvkrlilmtrgnvvivcamtaligtiaqldga gavtfllsipallplykalnmnkyllilllalsaaimnmvpwggpmarvaavlkaksvne lwyglipiqiigfilvmlfavylgfkeqkrikkaiernelpqtqdidvhklvevyerdqd vrfpvkgrartkswikwvntaltlavilsmliniappefafmigvslalvinfksvdeqm erlrahapnalmmaaviiaagmflgvlnetgmlkaiatnlikvipaevgpylhiivgllg vpldlltstdayyfavlpiveqtagqfgvpsvstaysmvigniigtfvspfspalwlaig laeanmgtyikyaffwiwgfaivmlviamlmgivti
345.	mentvkyrkfilpivvglliwaltpfkpdavdptawymfaifvatiiacitqpmpigavs iigftimvlvgivdmktavagfgnnsiwliamaffisrgfvktglgrrialhfvklfgkk tlglaysivgvdlilapatpsntaraggimfpiiksisesfgskpkdgsarkmgaflvft efqgnlitaamfltamagnplaqnlasstsnvhitwmnwflaalvpglvslivvpfiiyk iypptvketpnakswaenelatmgkialaekfmigifvvaltlwivgsfihidatltafi alalllltgvltwqdilnetgawntlvwfsvlvlmadqlnklgfipwlskslatslggls wpivlvililfyfyshylfasstahisamyaallgvaiaagapplfsalmlgffgnllas tthyssgpapilfssgyvtqkrwwtmnlilgfvyfiiwiglgslwmkvigif
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352.	mkhyltkfvamlitaamvcsfgllksqaaeqqsisdvysvitdaksalsnnsisndnkqk aieqvvsavkklsllednsesnavksdvrkledakandnqkdtlsqltksliayeeklask dagskiklllqqqvdakdaamtkaikdknkaeleslnnslnqiwtsnetvirnydanqygq ievallqlriaihkspldtakvshawttfksnidhvdkksntsandqyhvsqlndaleka ikaiddnqlsdadaalthfietwpyvegqiqtkdgalytkiedkipyyqsvldehnkahv kdglvdlnngikevvghsysfvdvmiiflreglevllivmtlttmtrnvkdkkgtasvig gaiaglvlsiilaitfvetlgnsgilresmeaglgivavilmfivgvwmhkrsnakrwnd mikmmyanaisngnlvllatiglisvlregveviifymgmigelatkdfiigialaivil iifallfrfivklipifyifrvlsififimgfkmlgvsiqklqllgamprhviegfptin wlgfypsyepliaqgayimvvailifkfkk

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444.

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579.	mshkilvsdpisedglqsilkhpefdvdiqtdlsendlvnmistydalivrsqtqvteri inaatnlkviaragvgvdninieaatlkgilvinapdgntisatehsvamllamarnipq ahqslrnkewnrkafrgvelygktlgvigagriglgvakraqsfgmkflafdpyltedka ksldiqiatvdeiaeksdfvtvhtpltpktrgivgssffnkakqnlqiinvarggiidet alieal dnnlidraaidvfeheppt dspliqhdkiivtphlgastveaqekvavsvseei ieiltkgnvehavnapkmdlskvdkttqsfiglsttigefaiqlldgapseikvkyagdl aqndtslitrtiitnilkedlgnevniinalailnqqgvtyniekqkkhsgfssyielel vndqdkikigatvfagfgprivrindysldfkpnqyqlvtchkdkpgivgqtgnllgshg iniasmtlgrndaggdalmilsidqqaseevikilnetsgfnkiistklti
580.	lkrnfinnliilliaimlslllkmlhvilpfmfgpilaallcvkvlklkirwpfwlsqig lillgvqigstftqqvikdisknwltivfvtillillaliiafffkkiaqvnletailsv ipgalsqmlvmaeenkkanilvvsltqtsrvifvvilvplisyffqdnhhemmhttmevp tlsqtlniwqiiilfsmvgiiyigmskinfptkqllapiivliiwmmtthltfsldhwll ataqliymiriglqianlmsdlkgriaiaiafqnimlivttfimiigihlitnesinelf lgaapggmsqivlvamatgadvamissyhifriffilfviapligyfinvklnnk
581.	vkktsriiafilliallftgmgmtyknvvknvnlgldlqggfevlfqvdplnkgdkidkk alqatsqtlenrvnvlgvsepkiqiedpnrirvqlagikdqaqarkllstqanltirdae dhvlmsgsdikqgsakqefkqetnqptvtfkvkskdkfkkvtekiskkrdnvmvvwldfe kgdsykkeakkqqegkkpkfisaasvdqpinsssveisggfngkkgveeakqiaellnag slpvdlkeiysnsvgaqfgqdaldktmfasivgialiylfmlgfyrlpglvaiialttyi yltlvafnfisgvltlpglaalvlgvgmavdaniimyerikdelrigrtlkqayskanks sfltifdsnlttvi aaavlfffgessvkgfatmlllgilmifvtavflsrgllsllvssn ffkkqywlfgvkkkdrhdinegkdvhdlktsyerlnfvklakplislsiliviigliiis ifklnlgidfssgtradiqsknaitqaqvektvksvglepdqiqingsgnknatvqfkkd lsreednklsakvksefgdnpqintvspliggelaknavtalilasigiiiyvslrfewr mglssvlallhdvfiiiaifslfrlevdltfiaavltivgysindtivtfdrvrenlhkv kvithtdqiddivnrsirqtmtrsintvltvvvvvvaililgaptifnfslalliglllsg vfssifiavplwgmlkkrqfkktknnklvvhkekksndekilv
582.	mgentkqdfnqkgqnfkftkkhrrllygsvflmatsaigpafltqtavftaqfyasfafa ilisiiidigaqiniwrilvvtglrgqeisnkvlpglgtiisiliafgglafnigniaga glglnamfgldvkwgaaitaifailifvsrsgqkimdvismilgivmilvvayvmvvsnp pygdalvhtfapehpfklilpiitlvggtvggyitfagahrildsgikgksylpfvnrsa vagilttgvmrtllflavlgvvvtgvtlssenppasvfqhalgpigknifgvvifaaams svigsaytsatflktlhkslln knnlivitfivistfvflfigkpvslliiagaingwil pitlgailiasrkksivgnyqhptwmlvfgiiavivtimtgifslqdlaslwkg
583.	vsnnnfkddfeknrqsinpdehqtelkeddktnenkkeadsqnslsnnsnqqfpprnaqr rkrrretatnqskqqddkhqknsdakttegsldd rydeaqlqqqhdksqqqnktekqsqd nrmkdgkdaaivngtsespehkskstqnrpgpkaqqqkrksestqskpstnkdkkaatga giagaagvagaaetskrhhnkkdkqdskhsnhendeksvknddqkqskkgkkaavgagaa agvgaagvahhnnqnkhhneeknsnqnnqvndqsegkkkggfmkillpliaaililgaia ifggmalnnhndskddqkianqskkdsdkkdgaqsednkdkksdsnkdkksdsdknadd dsdnsssnpnatstnnndnvannnsnytnqnqqdnanqnsnnqqatqgqqqshtvygqenl yriaiqyygegtqanvdkikranglssnnihngqtlvipq
584.	makgdqyqahtekyhdkkskksykpvwiiisfiilitilllptpaglpvmakaalailaf avvmwvteavtypvsatlilglmilllglspvqdlseklgmpksgdiilkgsdilgtnna lshafsgfstsavalvaaalflavamqetnlhkrlallvlsivgnktrnivigailvsiv laffvpsataragavvpillgmiaafnvskdsrlaslliitavqavsiwnigiktaaaqn ivainfinqnlghdvswgewflyaapwsiimsialyfimikfmppehdaieggkelikke lnklgpvshrewrlivisvlllffwstekvlhpidsasitlvalgiilmpkigvitwkgv ekkipwgtiivfgvgislgnvllktgaaqwlsdqtfglmglkhlpiiatialitlfnili hlgfasatslasalipvfisltstlnlgdhaigfvliqqfvisfgfllpvsapqnmlayg tgtftvkdflktgipltivgyilvivfsltywkwlglv
585.	mldfinhllsyqflnralitsilvgivcgtmgsiivlrglslmgdamshavlpgvalsfl fnipmfigalvtgmlaslfigfitsnsktkpdaaigisftaflasgviiislinsttdly hilfgnllaithqsfwttivtvlvillliifyrplmistfdatfsrmsglnttlihyfv mlllalvtvasiqtvgiilvvallitpastafliskqlyammviasiisvissiiglyfs yiynipsgativictfmiyivtlsitriknkqkrsalt
586.	lakllyklgkfiaknkwlsvigwlvilgviitplminspkfdsditmnglksldtndkis kefhqdsekasmkivfhsnknd glnnkdtkkdiedaldnirqnddyiqnisnpydsgqvn degdtaianvsyvvpqtglkdsskhiidkelkdvtdnhnvqiektqqgamnsepggtsei vgiivafvillitfgsliaagmpiisaiiglgssvgiialltyifdipnftltlavmigl avgidyslfilfrfkelkkkgvdtveaiatavgtagsavifagltvmiavcglslvgidf lavmgfasaisvlfavlaaltllpalisifhksikikdkptkskdpkdhswakfivgkpv iavivsliiililaaipvsgmrlgipddslkptdsseykayklisdnfgegyngqivmlvn tkdggskstierdlnnmrsdledidnvdtvskaqltdnnnyalftiipekgpnsqstenl vydlrdyhsqaqekydygteisgqsvinidmseklnnaipvfagvivvlaffllmivfrs ilvplkavlgfilslmatlgfttlviqhgfmgslfgientgpllaflpvitiglifglai dyelflmtrvheeyskt gdndhsirvgikesgpvivaaalimfsvfiafvfqddsaiksm gialgfgvlfdafvvrmtlipaltklfgkaswylpkwlgavlpnvdvegkaleednhhdt ssekghvndknseysrqdkdnyvyqndkrnynrnyndedynrsvhlnnhhdqhhrqhqyd nqrddidyeslytqdgdhthhdernyndrhyqdnydrnddyrhnnhdhqndnhdyhdsnf dkttnlykeltdsnidqdvlfkalmlyarennkgvydrynrssqhrhddelrd
587.	mnkkvehignqytsqenkkkqrqkmkmrvvrrrialfggillaiillvllviqrhnnd qdaverkeketefqkqqdeeialkeklnnlndkdyiekiarddyylsnkgevifrlpddk kssqsktsnekgn
588.	mkirltfiilailstiglvlvlakyptgphtinynepytvliaittivimalpalilgif nhlacriisailqisalmmwgflviislimgqivimlmasltilallvssivtlsvhpst sdkin

589.	mnkkllwsiigiviivvliiaafilkqvngsgskdsnaydtytvrketpislegkaspes vktynnnqsvgnflsvsvqdgqtvkqgeriinydtngnkrqqllnkvnqaqsqvnddyqk vnqspnnhqlqvkltqdqsalneaqqslsqydrqlndsmnasfdgkiniknndsdvgegqp ilqlissnpqinatitefdinkikegdevnvtvnstgkkgkgkilkidelptsydtsdds tassaqagaqgdseegtemttsnptinqptggksgetskykviigdldipvrsgfsmdak iplktkklpnnvltkdnnvfvvdknnkvhkreikiernngeiivkkglksgdkvlkspkg nlndgekvevss
590.	maettkifeshlvkqalkdsvlklypvymiknpimfvvevgmllalgltiypdlfhqesv srlyvfsifiillltlvfanfseqlaegrgkaqanalrqtqtemkarrikqdgsyemida sdlkkghivrvatgeqipndgkvikglatvdesaitgesapvikesggdfdnviggtsva sdwleveitsepghsfldkmiglvegatrkktpneialftllmtltiiflvviltmypla kflnfnlsiamlialavclipttiggllsaigiagmdrvtqfnilaksgrsvetcgdvnv lildktgtitygnrmadafipvksssferlvkaayessiaddtpegrsivklaykqhidl pqevgeyipftaetrmsgvkfttrevykgapnsmvkrvkeagghipvdldalvkgvskkg gtplvvledneilgviylkdvikdglverfrelremgietvmctgdneltaatiakeagv drfvaeckpedkinvireeqakghivamtgdgtndapalaeanvglamnsgtmsakeaan lidldsnptklmevvligkqllmtrgslttfsiandiakyfailpamfmaampamnhlni mhlhspesavlsalifnaliivllipiamkgvkfkgastqtilmknmlvyglggmivpfi giklidliiqlfv
591.	mivlrrlfqdrgaifaiaiitiyvvlgvlaplitfyepnhidtankfagiswshwlgtdh lgrdvltriiyairpsllyvfvaliisvvigailgfisgyfpgyidaiimricdvmlafp syvvtlalitlfgmgveniiiafiltrwawfcrvirtsvmqyieadhvkfakvigmndlt iirkhilpltftdiaiiasssmcsmilqmsgfsflglgvkaptaewgmmlnearkvmfth pgmmmttgvaiviivmafnflsdalqmaidprmsakekrlalkkgvkardta
592.	mkgamswpflrlyiltlmffsanailnvfiplrghdlgatntvigivmgaymltamlcrp wagqiiarigpikvlriillinamalvlygftglegyliarimqgvctaffsmslqlgii dalpekyrsegvslyslfstipnllgpliavgiwhvenmsifaivmifiavtttlfgyrt tfantqkevspkdevlpfnamtvyvqffknkalfcsgmimilssivfgamstfiplytvr egfanagifltiqaitvviarfylrkyvpsdglwhhrfmmivltllmvasvivafgphiv sifvyisaifigitqalvyptlttylsfvlpkigrnmllglfiacadlgislggvlmgpi sdtvgfkwmyilcallvtiamtlskirqrqsvskas
593.	vgstvkyrkfilpivvgliiwaltpikpdalndqawfmfaifvstiiacitqpmtigavs iigftimilvgivdtktavqgfgnssiwliamaffisrgfvktglgrrialqfvklfgkk tlglayslvgvdlilapatpsntaraggimfpiiksisesfgssprdgserkmgaflift efqgnlitsamfltamagnpiaqslaektahvqitwmmwfvaaiipglislivvpfiiyk lypptvketpnakkwateqleemghmsiaeklmvgifiialalwvlgsfinvdatltafi alaillltgvlawsdilnetgawntlvwfsvlvlmaeqlnklgfipwlskliaqglngfs wpivlvllilfyfyshylfasatahvsamyaallgvavasgapplfsalmlgffgnllas tthyssgpapilyaagyvtqkrwwtmnivlgivyfiiwigvgslwmkligmm
594.	mkdnkmlfiifmigtftvgmaeyvvtglltqiaddmkvsissagllsvyaisvaligpl mriitlkvhahrllpilvaifiisnlvgmlapnfnvlllsrlmsaamhapffgvcmsvaa tvappakktqaialvqagltiavmlgvpfgsflggfanwrvvfgfmivlaiitmlgmikf vpnvslsaeaniskeltvfkmphiliviaiivfgysgvfttytfmepmirdfspfkivgl tvclfmfglggvignlitgmvpedkltknlyltfllfvtiilfvtviqmsilaliicfl fgfgtfgttpllnskiilsgkeapllastlaasifnvanflgaiigsillsiglpyiqit lisggiivlgmllnlvnqlyekkhitfneys
595.	MAVKVAINGFGRIGRLAFRRIQEVEGLEVVAVNDLTDDDMLAHLLKYDTMQGRFTGEVEV VDGGFRVNGKEVKSFSEPDASKLPWKDLNIDVVLECTGFYTDKDKAQAHIEAGAKKVLIS APATGDLKTIVFNTNHQELDGSETVVSGASCTTNSLAPVAKVLNDDFGLVEGLMTTIHAY TGDQNTQDAPHRKGDKRRARAAAENIIPNSTGAAKAIGKVIPEIDGKLDGGAQRVPVATG SLTELTVVLEKQDVTVEQVNEAMKNASNESFGYTEDEIVSSDVVGMTYGSLFDATQTRVM SVGDRQLVKVAAWYDNEMSYTAQLVRTLAYLAELSK
596.	vkrlknfilgllivaivgfllfmyiddsriqsyqdyflqfnwfqplliglaglliligli lvlsifkpthrkpglyknfddghiyvsrkavektiydtiakydqvrqpnvvsklynkknk sfidikadffvpnhvqvksltesiradiksnvehfteipvrklevnvrdqktsgprvl
597.	msflrkhteiifsyiigivslftgliifinlplikqfkgdkkvdthvhnvweflnaffae iikvmskfiggfpitsaiviivfgilvmllghtlfrtikydydisifflvigimyfiitl llmtqvygffaivfiipftvhigyivykdelnqdnrknhymwiivtygmsylitqislyg ridaneiesidilsvntffiimwllgqmaiwnflflrrslpltkeelgeeepelsrtnkg nvsnqtkvhlkqlqnktteyarktrrsvdldkirakrdkfkqkinsivdiqeddipnwmk kpkwvkpmyvqlfcgviilffaflefnnrnalfltgewelsqtqyvvewvtllllfiii iyiattltyylrdkyyylqlfmgsilffkfltefinimvhglllsifitpilllmliami vayslqlrek
598.	mqqettswykqewfivlsllfifplglflmwkfskwpsiartiitvaisvivlasityyg nlqmivpatsnsnnetkettennvndkdernhktaveetktnydstkentkepgkenesa trlensalekaksyyddfhmsklgiyditlseygekfdkedaqyaidhleadyeknalek aksyakdmhmsndsiydllvsnygekfteseakyaiehldn